COMPENSATORY HYPERTROPHY OF THE KIDNEY OF THE YOUNG RAT WITH SPECIAL EMPHASIS ON THE ROLE OF CELLULAR HYPERPLASIA

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EIGHT FIGURES

That the remaining kidney enlarges following unilateral nephrectomy has been known for a considerable time. The investigations of Arataki ('26), Moore ('29), and others indicate clearly that this enlargement is accomplished by an increase in the size of the nephrons present or normally developing at the time operation; no new nephrons are formed. In respect to the nephrons, then, the enlargement of the kidney is an hypertrophy. However, the mechanism of this hypertrophy has not been satisfactorily explained.

The rapidity of the increase in weight of the remaining kidney following unilateral nephrectomy led Morel and Verliac ('13) to postulate on theoretical grounds that there was an initial stage of pseudohypertrophy in which edema predominates. They suggested that the pseudohypertrophy, lasting as long as 10 days, might cause the true hypertrophy which follows. They admitted, however, that the two processes must overlap, thus making it difficult to determine the start of the second phase. Jackson and Levine ('29) obtained data on the gross enlargement of the kidney which they believed indicated an initial, transient stage of pseudohypertrophy of less than one week's duration. Others, on the other hand,

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have reported no evidence of a pseudohypertrophy (Moise and Smith, '26).

The existence of cellular hyperplasia in addition to cellular hypertrophy in the enlargement of the kidney has also been questioned. From a review of the literature it almost appears that the kidney cell differs from many other epithelial cells in that it can not be easily stimulated to divide. Galeotti and Villa-Santa ('02) report finding no evidence of cellular hyperplasia having played any role in the enlargement of the kidney. Oliver and Lund ('33), on the other hand, concluded that cellular hyperplasia occurred since they found more nuclei in cross sections of enlarged proximal convoluted tubules than in normal tubules. However, they make no mention of having observed any mitotic figures. Saphir ('27) states definitely that he saw no mitotic figures in his study of the enlargement of the kidney of the rabbit.

The effects of thyroxin and testosterone on the kidney have also been studied histologically with similar rather surprising results. Korenchevsky and Hall ('44) report that non-toxic doses of thyroxin cause enlargement of the liver by cellular hyperplasia whereas the enlargement of the kidney caused by the same treatment was apparently due to cellular hypertrophy. Testosterone also causes enlargement of the kidney, and again both Korenchevsky and Ross ('40) and Lattimer ('42) report that mitoses are scarce.

In view of the above reports it seemed worthwhile to reinvestigate rather systematically the enlargement of the remaining kidney following unilateral nephrectomy in an effort to obtain direct evidence as to whether or not cellular hyperplasia within the individual nephrons played a significant role in the process, and, if so, when and where this hyperplasia occurred. It was thought that this information plus additional data on the increase in weight of the remaining kidney after unilateral nephrectomy and on the time of appearance of cellular hypertrophy would also shed light on the problem of the existence of an initial pseudohypertrophy.

I wish to express my sincere appreciation to Professor Alden B. Dawson for suggesting this problem and for his advice and guidance throughout the course of the investigation.

MATERIALS AND METHODS

Because others had apparently failed to find dividing cells in the enlarging kidney it was felt advisable to make this study on young rats in which the nephrons were still growing as evidenced by the presence of numerous mitotic figures. It was assumed that cells which were normally still dividing could be more easily stimulated to greater mitotic activity than could cells which had practically ceased all such activity. It also had been demonstrated (MacKay, MacKay and Addis, '32) that compensatory hypertrophy, as measured by weight increase, becomes less as age advances. Therefore cellular hyperplasia might be more readily demonstrated in young animals if indeed it did occur.

Litters of 4 or 5 healthy male rats of the inbred strain maintained in the Biological Laboratories of Harvard University were used in the present investigation. Two animals in each litter served as controls; the other two or three animals were unilaterally nephrectomized at two weeks of age. The right kidney was removed from the experimental animals by a dorsal incision made under ether anesthesia. Care was taken not to disturb the right adrenal gland. After closing the body wall the wound was covered with celloidin to prevent the mother rat, which was left with the young for one week after the operation, from reopening the incision. Animals so treated recovered quickly and showed no ill effects from the operation. Litters were then sacrificed daily for the first 10 days and at irregular intervals thereafter to the 56th day after operation. In view of the demonstrated diurnal periodicity of mitosis in the kidney of the rat (Blumenfeld, '38), all rats were killed between two and three o'clock in the afternoon, at the peak of daily mitotic activity.

After weighing and killing each rat, the left kidney was removed, weighed on a torsion balance, and a median trans-

verse slice was fixed in Helly's solution. Following the usual paraffin technique, serial transverse sections were cut at 7 μ and stained with Ehrlich's haematoxylin and eosin.

Since these experiments were performed on growing rats in which both body and kidney weight are changing normally, comparisons of kidney weight were made on the basis of the ratio of kidney weight to body surface. This relationship was chosen because MacKay and MacKay ('27) have shown that in normal rats between 35 and 760 days of age, kidney weight has a very nearly constant relation to body surface whereas it decreases relative to body weight and increases relative to body length. The skin area of each rat was computed from the formula $S = kW^{2/3}$ in which S = skin area in cm^2, k = Meeh's constant, and W = weight in grams. Meeh's constant has been experimentally determined for the white rat (Carman and Mitchell, '26) to equal 11.36.

Microscopic measurements of two types were made on most of the kidneys. First, the diameter of 25 proximal convoluted tubules located throughout the entire width of the cortex of each kidney was measured under an oil immersion objective with the aid of an ocular micrometer inserted into a 10 × ocular. The distance between micrometer divisions measured 0.77 μ. Only those tubules which had been sectioned perpendicularly to their long axis, as indicated by their circular outline, were measured.

Second, and of particular interest in this investigation, the degree of cellular hyperplasia in each kidney was determined with the aid of a 10 × ocular into which a glass disc marked off in squares was inserted and a high dry objective, giving a field the area of which was 0.0654 mm^2. Mitotic figures in the cells lining the tubules were counted in 10 such fields chosen at random in the cortex, outer medulla and inner medulla of each of 10 sections. The sections used in this analysis were separated from each other by at least 63 μ, thereby insuring that the same division figures were not counted twice. Thus the number of mitoses per 100 fields

with a total area of 6.54 mm^2 was determined in each of the three zones of each kidney

OBSERVATIONS

Although in the present investigation the microscopic anatomy of the kidney is of primary interest, attention might be briefly turned to the gross enlargement of the remaining kid-

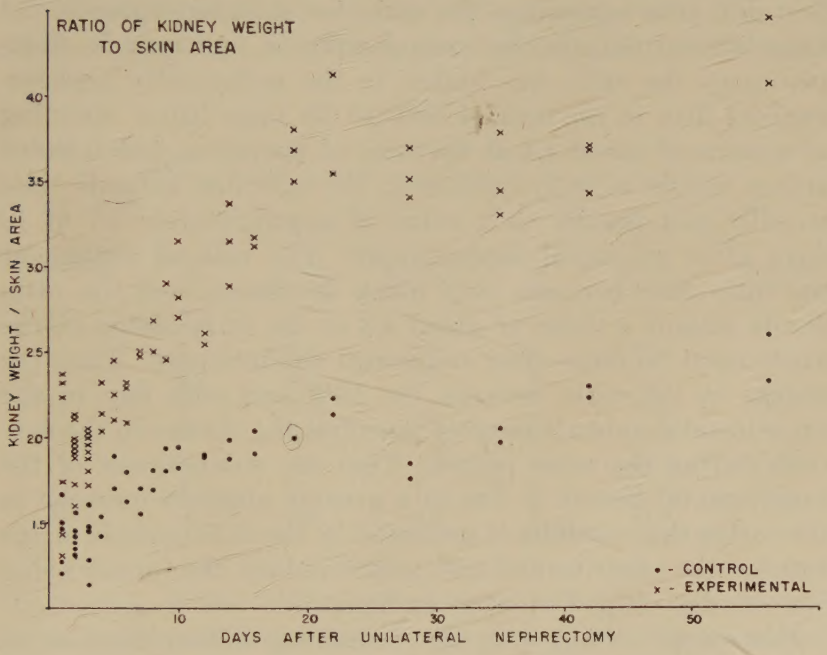

Figure 1

ney following unilateral nephrectomy. The ratio of kidney weight in milligrams to body surface in square centimeters for each rat is shown in figure 1. It is apparent that during the first 10 days of the experimental period, when the rat is 14 to 24 days of age, the weight of the kidney of the normal animal is increasing quite rapidly relative to the skin area. The ratio rises from approximately 1.4 to 1.9. Between the 10th and 56th day of the experiment the ratio of kidney

weight to skin area in the control animals shows a further rise to a value of approximately 2.4. Because of the small number of animals sacrificed between the 16th and 56th day of the experiment the value of the ratios during this time can be considered as only approximate. In the experimental animals the response of the remaining kidney to unilateral nephrectomy is reflected immediately in the ratio of kidney weight to body surface. In every case except two on the first day after operation, the ratio has a higher value in the experimental than in the control animals, and even in these two cases the ratio was higher in the unilaterally nephrectomized than in the control rats of the same litter. Starting at a value of about 1.4 at the time of operation, the ratio of kidney weight to body surface in the operated animals rises steadily and rapidly to a value of approximately 3.7 at 19 days after unilateral nephrectomy. The rate of change of the ratio then becomes very much decreased, and the ratio finally attains a value of about 4.3 at the termination of the experiment 56 days after unilateral nephrectomy. Thus the change in the ratio between the 19th and 56th day in the experimental animals roughly parallels the change in the controls during the same period. That the enlargement of the experimental kidney is due to a greater absolute increase in the cortex than medulla is indicated by the difference in shape between the experimental and control kidney, the former being more wedge shaped in cross section.

Microscopic changes in the remaining kidney also occur very shortly after unilateral nephrectomy. The mean diameter and standard deviation of 25 measurements of proximal convoluted tubules distributed throughout the cortex of the kidney of each of a number of the animals is given in table 1. While there is considerable variation, the measurements seem to indicate that following operation there is an almost immediate increase in the diameter of the tubules of the experimental animals. That the increase is due to cellular hypertrophy rather than simply dilation of the tubules is indicated by the apparent lack of any change in the width

of their lumina. Although the diameter of the proximal convoluted tubules increases with age in both the control and experimental animals, this difference between the two groups remains more or less constant throughout the course of the experiment.

A final aspect of the enlargement of the remaining kidney following unilateral nephrectomy which has been investigated in the present study involves a direct determination of cellular hyperplasia within the individual nephrons. Figure 2 shows the number of mitoses per 100 fields in the tubules of

TABLE 1

Mean diameter of the proximal convoluted tubules in control and enlarged kidneys based upon 25 measurements in each kidney

DAYS AFTER OPERATION	CONTROL			EXPERIMENTAL		
	Number of rats	Mean diameter	Standard deviation	Number of rats	Mean diameter	Standard deviation
		μ			μ	
1	4	20.7	2.7	6	23.4	3.7
2	6	20.4	3.0	9	22.4	3.0
3	3	21.9	3.4	7	23.9	3.0
6	2	21.6	2.5	3	23.8	3.0
9	2	27.3	3.3	2	28.6	3.9
22	2	31.3	4.1	2	35.6	3.9
56	2	32.6	3.8	2	34.9	5.0

the cortex of the kidney of each control and experimental animal. Mitoses were seen in all parts of the nephron and in the connective tissue, but the great majority of mitoses in the cortex were in the convoluted tubules. They are very rare in Bowman's capsule and relatively infrequent in the connective tissue. It will be seen that at the time of operation, growth of the kidney tubules by cell division is still quite active; the cortex of the control kidneys showing approximately 200–300 mitoses per 100 fields. With advancing age, the number of mitoses in the cortex of the control animals gradually decreases and by 22 days after the time of operation has reached a level of only about 25 cell divisions per 100

fields. Five weeks later, 56 days after the start of the experiment, the mitotic rate in the cortex has still further decreased to approximately 12 mitoses per 100 fields.

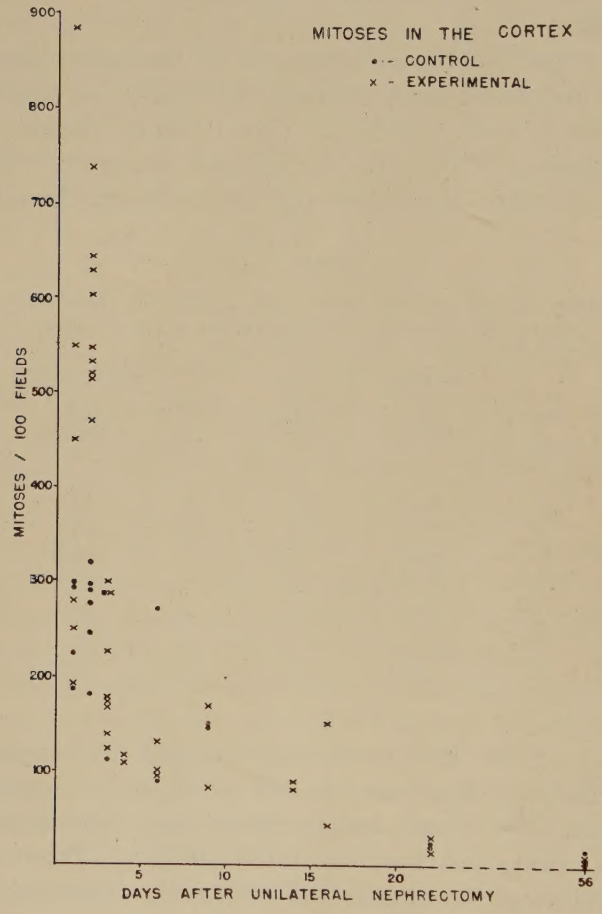

Figure 2

Mitotic activity in the cortex of the experimental kidney shows a very rapid response to unilateral nephrectomy. Although three of the 6 animals killed one day after operation show approximately the same number of mitoses in the cortex as is shown in the cortex of the control kidneys, this zone of

the kidneys of the other three animals shows many more dividing cells than the controls. Eight hundred and eighty-four mitoses were counted in 100 fields in the cortex of one of the animals of the latter group. On the second day, the cortex of each of the experimental animals shows two to three times as many mitoses as the controls, one animal showing 738 division figures in 100 fields. Figures 5 and 6 are sections through the cortex of this animal and show many mitoses in the convoluted tubules. Figure 6 also shows a mitotic figure in the thick segment of Henle's loop. On the third and subsequent days it is evident that the number of mitoses in the cortex of the experimental kidney is again at the level found in the control animals. Thus, in some animals on the first and in all animals on the second day after operation, and on those days only, there is a marked increase in the number of mitoses in the cortex of the kidneys of the unilaterally nephrectomized animals.

In the adjoining outer medulla of the control kidney there are approximately 200–400 mitoses per 100 fields at the start of the experiment (fig. 3). This number decreases quite rapidly to about 70 mitoses at 9 days, 17 mitoses at 22 days and 7 mitoses per 100 fields at 56 days after the beginning of the experiment. In the unilaterally nephrectomized animals the number of mitoses in this zone of the remaining kidney of two animals sacrificed on the first day after operation shows a possible slight increase as compared with the controls. On the second day the number of mitoses has increased to between 400 and 600 mitoses per 100 fields. Figure 7 is a photomicrograph of a portion of the outer medulla of an experimental kidney two days after unilateral nephrectomy. On the third day after operation the number of mitoses in the outer medulla of the remaining kidney has returned to the control level in three-quarters of the animals. The other two animals still show increased numbers of dividing cells. After the third day there is little difference in the degree of mitotic activity between control and experimental animals.

Finally, the number of mitoses per 100 fields in the inner medulla of the control and experimental kidneys is given in figure 4. This zone shows considerably less mitotic activity than either of the other two zones of the kidney. In the controls, there are fewer than 75 mitoses per 100 fields at the

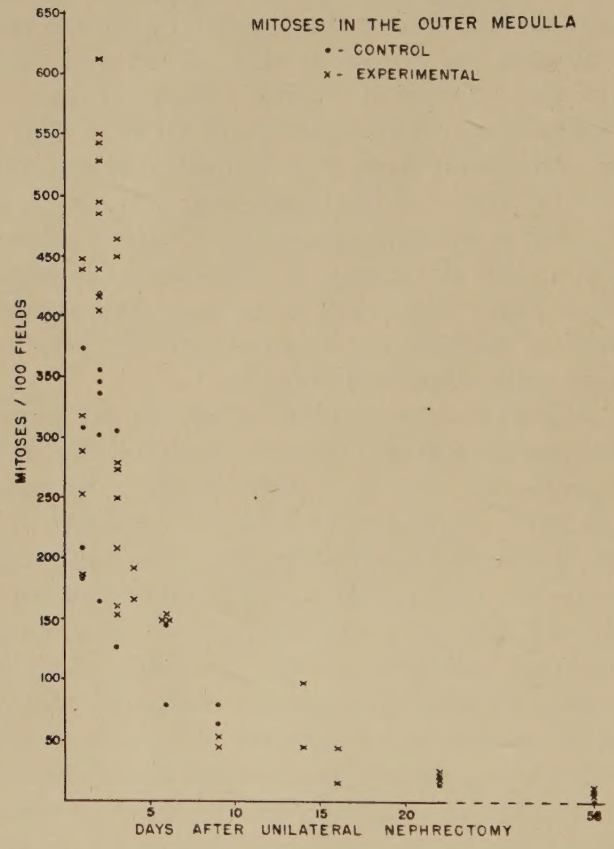

Figure 3

start of the experiment. Even this rate diminishes rapidly so that no more than 4 mitoses were counted in 100 fields on the 6th and 9th days after operation. No mitoses were seen in 100 fields in this zone of the control kidneys 22 and 56 days after the start of the experiment. The condition in this zone

of the experimental kidneys is similar to that already described for the other zones of the kidney. On the first day after operation there is no significant difference between the control and experimental animals. Two days after unilateral nephrectomy, however, a marked increase in mitotic activity appears in the remaining kidney of about half of the animals.

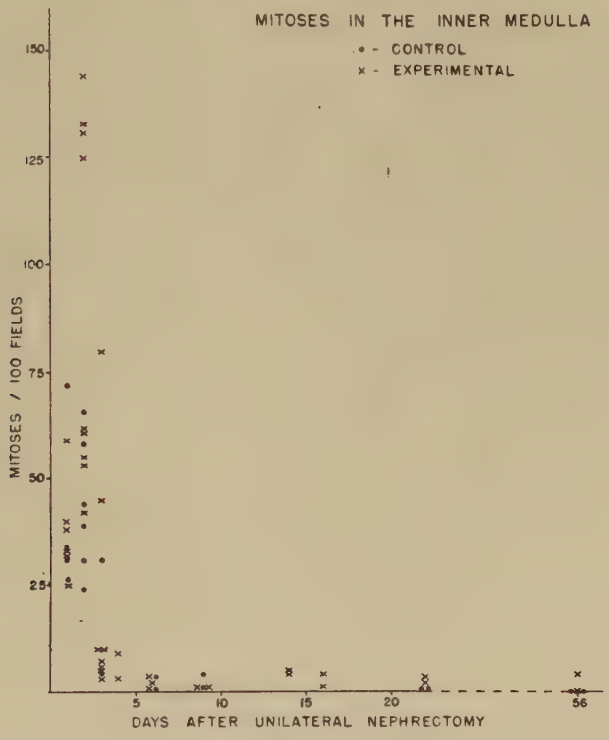

Figure 4

In the inner medulla of these kidneys between 125 and 144 mitoses were counted in 100 fields. A photomicrograph of a portion of the inner medulla of one of these kidneys is shown in figure 8. On the third day the number of dividing cells is drastically reduced in all except one kidney which still shows possible evidence of hyperplasia. After the third day the number of mitotic figures in this zone of the experimental kidney is again equal to that of the control kidneys.

Thus unilateral nephrectomy is followed by a marked increase in the number of mitoses in all three zones of the kidney on the second day after operation. On the first and third days some of the kidneys show increased mitotic activity, but on subsequent days the degree of mitotic activity in the experimental kidneys is the same as in the control kidneys.

DISCUSSION

That the remaining kidney would enlarge following unilateral nephrectomy might perhaps be anticipated before any investigation of the subject. The animal is suddenly forced to excrete with one kidney everything which it formerly had excreted with two. The fact that the kidney becomes heavier has been demonstrated many times in the past. Concerning the rate, extent and manner of enlargement, however, there has been considerable debate.

Among the investigations of this subject are several which are concerned with the enlargement of the remaining kidney following unilateral nephrectomy in young as compared with old animals. Carnot ('13), in rabbits, and Jackson and Shiels ('27), in rats, reported a greater relative enlargement in young than in old animals while Arataki ('26), in rats, reported the opposite finding. Braun-Menéndez ('46), on the other hand, found no significant age differences in rats, except possibly a more rapid response in adults than in young animals. In a more extensive study than any of the preceding, MacKay, MacKay and Addis ('32) found that, measured by an increase in weight, the compensatory hypertrophy of the kidney of the rat decreases rapidly from 5 days to 60 days of age and then shows a slow diminution throughout adult life. Addis and Lew ('40) also demonstrated a greater hypertrophy in rats operated upon at 30 days as compared with those unilaterally nephrectomized at 70, 110, and 120 days of age. They report a still greater relative enlargement of the remaining kidney following unilateral nephrectomy of the newborn. In the present study the rats were unilaterally

nephrectomized at 14 days of age, and it was found that 19 days after operation the remaining kidneys were approximately 80% heavier than the control kidneys relative to body surface. Thus the results of the present experiment, when compared with the results of others, substantiate the claim of a greater hypertrophy following unilateral nephrectomy in young as compared with old animals.

Another debatable aspect of the enlargement of the remaining kidney following unilateral nephrectomy is that of the presence or absence of an initial "pseudohypertrophy," i.e., a temporary enlargement due to an influx of fluid which precedes the true hypertrophy or increase in renal tissue. No evidence for a transient pseudohypertrophy was found by Moise and Smith ('26), Smith and Moise ('27), or Jackson and Shiels ('27). Braun-Menéndez ('46) similarly reports that the true increase in the remaining kidney starts immediately and is progressive although dry weight determinations indicated that there was a considerable accumulation of fluid during the first days after operation. Jackson and Levine ('29), however, report that in a small group of rats removal of the right kidney was followed by a rapid increase in the weight of the remaining kidney which reached an apparent hypertrophy of 34.89% in three days. This was followed by a decrease in the percent hypertrophy during the remainder of the first week and subsequently by an irregular progressive renal hypertrophy.

The data presented in this paper give no indication of a transient pseudohypertrophy such as that described by Jackson and Levine. The latter workers state that their data must be interpreted with caution since they killed only one control and one unilaterally nephrectomized rat each day, and it appears to this author that their results can be explained on the basis of individual variation. The peak which they obtained on the third day (34.89% hypertrophy) is made more prominent than it might otherwise be by the fact that they found only 12.90% hypertrophy in the animal which they killed on the 4th day. The latter figure seems from the pres-

ent work to be quite definitely near the lower limit of variation. The appearance of cellular hypertrophy 24 hours after operation and of cellular hyperplasia 24 to 48 hours after operation is further evidence against a pseudohypertrophy. It appears from the present work that the essential changes which lead to the enlargement of the remaining kidney following unilateral nephrectomy begin immediately after recovery from the shock of the operation.

The length of time required for the enlargement of the remaining kidney following unilateral nephrectomy to reach a maximum has also been the subject of disagreement. Thus Moise and Smith ('26) report that the hypertrophy is progressive to 120 days while MacKay, MacKay and Addis ('32) and Addis and Lew ('40) state that it is complete at 40 days. The graphs of the latter indicate a rapid initial increase which appears to be nearly complete at 20 days after operation. Braum-Menéndez ('46) reports a maximum in 8 to 20 days. In the present series of rats results similar to those of MacKay, MacKay and Addis and of Addis and Lew were obtained, namely a rapid initial increase to 20 days after operation and a possible though very slight further increase thereafter. Why Moise and Smith should have obtained such a different response from that obtained by the other investigators is difficult to explain.

That both the cortex and medulla are involved in the enlargement of the remaining kidney following unilateral nephrectomy is indicated by various workers. Arataki ('26), however, reports that in unilaterally nephrectomized rats, the relations of the two zones are very nearly maintained in all cases while Jackson and Shiels ('27), in nephritic rats, and Oliver ('24), in unilaterally nephrectomized rabbits, report a greater relative increase in the cortex than in the medulla. In the present study it is clear that both zones are involved though the cortex showed a greater mitotic response than the medulla. The shape of the kidney, too, indicated a greater absolute increase in mass of the cortex than of the medulla.

In studies of the microscopic changes involved in the enlargement of the remaining kidney following unilateral nephrectomy there has been general agreement that the nephrons increase in size. That the enlargement is the result of hypertrophy of the cells, particularly those of the convoluted tubules, and of dilation of the tubules is reported by Oliver ('24) in rabbits, Jackson ('25) in nephritic rats, and Peters ('29) in mice. There is very little evidence, however, that cellular hyperplasia is involved in the enlargement of the nephrons. In his study of the glomeruli in the remaining kidneys of unilaterally nephrectomized rabbits, Saphir ('27) states that he saw no mitotic figures. Similarly, Galeotti and Villa-Santa ('02) found no variation in the number of nuclei in cross sections of tubules of normal and enlarged kidneys of dogs and rabbits. In cross sections of the enlarged proximal convoluted tubules in kidneys from humans with Bright's disease, however, Oliver and Lund ('33) found more nuclei than in cross sections of normal tubules so concluded that hyperplasia "played a considerable part" in the increase in length and diameter of this portion of the tubule. Since they studied pathological specimens in which enlargement had probably occurred some time previously, it is not surprising that they make no mention of seeing mitotic figures. Bollman and Mann ('35) greatly increased the compensatory hypertrophy of the remaining kidney of the dog after unilateral nephrectomy by transplantation of the ureter of the remaining kidney into the duodenum. They killed their animals three to 9 days after operation and report that many mitotic figures were observed in the tubular epithelium. Unfortunately, they give no hint as to the number or location of the dividing cells nor do they indicate the relative numbers at different times after operation.

In the present investigation the diameter of the convoluted tubules was measured, and it appeared that enlargement of the cells accounted in large part for the increase in diameter of this part of the tubule following unilateral nephrectomy. Although other parts of the tubule were not measured, it is

also clear that hypertrophy is important in the enlargement of the renal corpuscles. The distribution of mitoses in the kidney indicates that cellular hyperplasia occurs in all parts of the tubule, though it is more active in some parts than in others. Recalling that the only parts of the nephron in the inner medulla are the collecting tubules and thin loops of Henle, it is clear that either or both of these parts of the nephron respond to removal of the other kidney by cell division. An analysis of the distribution of dividing cells indicates that the majority of the mitotic figures in this zone are in the collecting tubules. In the outer medulla one again finds collecting tubules and thin limbs of Henle's loop and in addition the thick portions of Henle's loop. This zone in the control kidney shows many more mitoses than the inner medulla, thus indicating that it is growing more rapidly than the latter at the time of operation. After removal of one kidney this zone of the remaining kidney shows a greater absolute increase in the number of mitotic figures than was found in the inner medulla. Since the thick limb of Henle's loop is the only part of the nephron not present in the inner medulla but present in the outer medulla, it appears likely that the cells of the thick limb of Henle's loop divide in considerable numbers following unilateral nephrectomy. Passing outward to the cortex, which shows the greatest mitotic activity, one again finds collecting ducts and Henle's loops. In addition to these and making up the greater proportion of this zone are the renal corpuscles and convoluted tubules. Although occasional mitotic figures were seen in Bowman's capsule, it is clear that the great increase in the number of mitoses reported in the cortex is due to the fact that many of the cells of the convoluted tubules are dividing. On the basis of this analysis and the reports in the literature it seems certain that the greatest anatomical response to unilateral nephrectomy occurs in the renal corpuscles and convoluted tubules. It appears that the glomeruli and Bowman's capsule become considerably enlarged, due primarily to an increase in the size of the cells of the capsule and of the capillary

tufts. Mitosis seems to play a minor role in this enlargement. In the enlargement of the convoluted tubles both hypertrophy and hyperplasia are important. In the other parts of the tubule there is a lesser response to removal of the other kidney though there is a significant hyperplasia and probably some hypertrophy and dilatation.

That the marked hyperplasia of the cells of the nephron on the second day after unilateral nephrectomy is not particularly unusual is shown by comparison with the regeneration of other organs. Studies of the remaining portions of the liver of the dog (Fishback, '29) and rat (Higgins and Anderson, '31) following the surgical removal of from one-fifth to three quarters of the hepatic tissue indicate a very rapid restoration of the lost tissue with marked cellular hyperplasia occurring during the first week. Quantitative studies of cell growth in the regenerating liver of the rat by Brues, Drury and Brues ('36) show that at the end of the second day following removal of 69.4% of the liver the number of cells in the residual liver represented an increase of 64% of the preoperative number of cells remaining in the body. There had been no significant increase during the first 24 hours, and the increment the third day was reduced to 26% which became progressively smaller during the remaining 9 days of the experiment. In a correlative study Brues and Marble ('37) determined that mitotic activity begins very rapidly after the 24th hour following operation though the rate falls markedly during the succeeding days. In necrosis and repair of the liver following prolonged chloroform anesthesia, too, mitotic figures are reported by Whipple and Sperry ('09) as being most numerous on the second day.

The enlargement of the remaining adrenal gland of adult female rats following unilateral adrenalectomy is reported to be due entirely to hypertrophy of the cells of the cortex (MacKay and MacKay, '26). It thus appears that the adrenal gland differs markedly from the kidney. If, however, one adrenal gland is removed and the other enucleated, the cortex

of the latter regenerates with mitosis starting on the second day after operation and continuing very actively during the following 4 days (Baker and Baillif, '39). Bilateral autotransplantations of adrenal glands to the ovaries in adult female rats also show that following an initial degeneration of everything except the capsule and a narrow rim of zona glomerulosa, centers of proliferation appear in the capsule and among viable cortical cells on the third day after operation (Ingle and Higgins, '38). When one considers the fact that this is transplanted tissue, the appearance of mitotic activity on the third day seems all the more rapid.

Thus cellular hyperplasia in both the regenerating liver and adrenal cortex begins 24 to 48 hours after operation, quickly reaches a peak and quickly subsides. It appears, then, to be very similar to the marked hyperplasia of the cells of the nephron after unilateral nephrectomy.

Finally, the results presented here must be considered in relation to the work of Blumenfeld ('44) in which he determined that the rate of mitosis in the renal cortex of normal young rats varies inversely with the volume of urine excreted. The mitotic activity in the submaxillary salivary glands of these same animals was also found to vary inversely with the amount of food eaten. The results of this investigation he cites as direct evidence for the theory that function restrains growth.

In the experiments reported here, however, it is clear that increased mitotic activity is an almost immediate response to the increased demands placed upon the remaining kidney. Reports in the literature indicate that the same is true in the case of compensatory hypertrophy of the liver. In both the kidney and the liver mitosis seems to occur in any cell; there is no evidence for the presence of reserve cells. Thus, although the results of the above investigation by Blumenfeld are most easily interpreted as he has done, it appears that extraordinary functional demands stimulate rather than inhibit mitosis.

SUMMARY AND CONCLUSIONS

In the present study two or three rats of litters of 4 or 5 males were unilaterally nephrectomized at the age of 14 days, the other two males in each litter serving as controls. Animals were killed daily during the first 10 days after operation, and at irregular intervals thereafter until the termination of the experiment 56 days after operation.

The weight of the remaining kidney relative to the calculated body surface showed a rapid initial increase which was progressive to approximately 20 days after operation. There seemed to be no further significant compensatory hypertrophy between the 20th and 56th days.

Measurements of the diameter of the convoluted tubules revealed that they become enlarged within 24 hours after operation. The fact that their lumina show little if any difference as compared with the controls indicated that the enlarged diameter is the result of cellular hypertrophy.

Counts of the number of mitoses per one hundred fields in the cortex, outer medulla and inner medulla demonstrated that cellular hyperplasia in the individual nephrons plays a significant role in the enlargement of the remaining kidney, though its action is largely restricted to the second day after operation. Significantly increased numbers of mitotic figures were observed in all three zones of the remaining kidney on this day, the greatest absolute increase occurring in the cortex.

The curve of enlargement of the remaining kidney in the present series of animals showed no evidence of an initial transient period of pseudohypertrophy. The fact that cellular hypertrophy is evident 24 hours after operation and that the increased mitotic activity is almost entirely restricted to the second day after operation indicates that the essential response of the kidney is almost immediate.

The early appearance of mitotic activity in the enlarging kidney possibly indicates that extraordinary functional activity stimulates mitosis.

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PLATE 1

EXPLANATION OF FIGURES

Transverse sections through the surviving kidneys of rats sacrificed on the second day after unilateral nephrectomy. Magnification × 450.

5 Cortex. Note particularly that several of these mitoses are in the same tubule.

6 Cortex. In addition to scattered mitoses in the convoluted tubules, one cell (slightly below and to the left of center) in the thick limb of Henle's loop is seen to be dividing.

7 Outer medulla. Shows scattered mitoses most of which are in the collecting ducts.

8 Inner medulla. Shows scattered mitoses in the collecting ducts.

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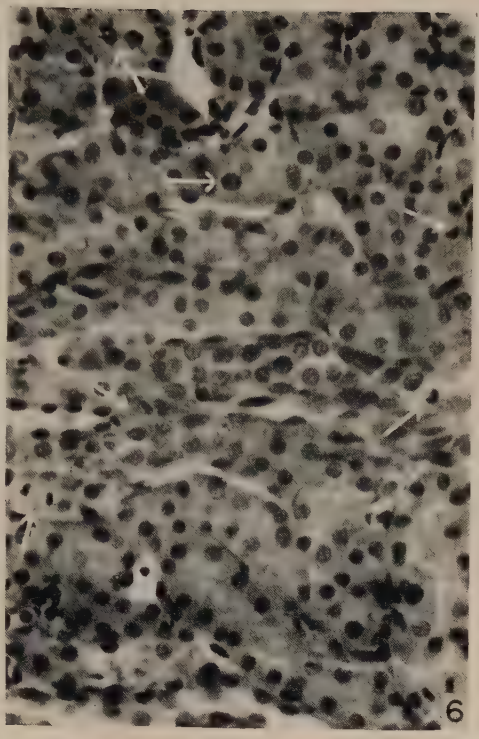
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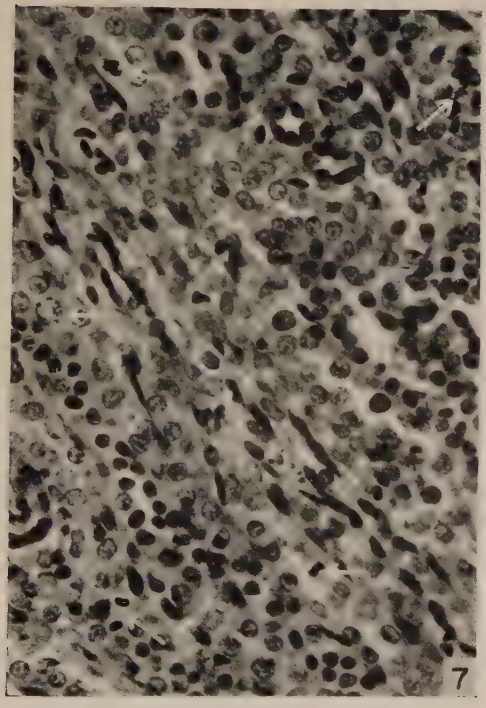
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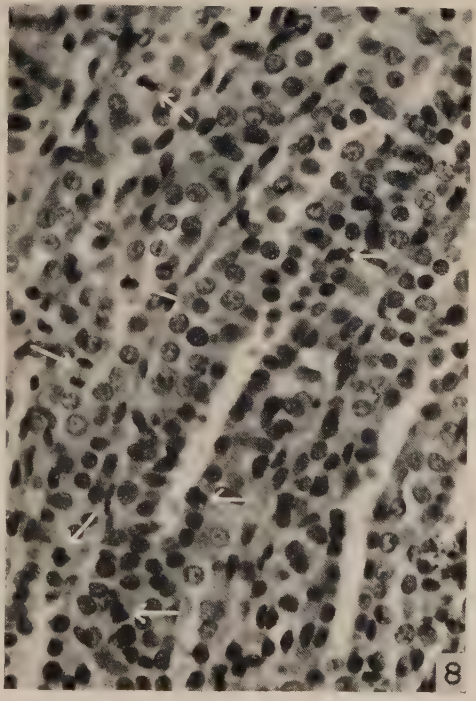
8

THE PRENATAL GROWTH OF THE HEART AND THE LUNGS IN THE DOG

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FOUR FIGURES

The prenatal growth of the heart and the lungs has been reported for man and for some of the laboratory animals, but no record has been found for the dog. More work has been done on the heart growth than on the growth of the lungs. The earlier papers on the development of these two organs have been reviewed (Latimer, '42 and '43) and this literature will not be repeated.

This paper will present the increase in weight of the heart and of the lungs and trachea taken together, with reference to body weight and to body length.

MATERIAL AND METHODS

The hearts and lungs from 156 fetal and 24 newborn dogs were used in this study. Four of the specimens were too small for positive sex determination by gross methods. The remaining 176 were evenly divided as to sex. There were 12 male and 12 female newborn dogs. There were 30 litters of the fetal dogs ranging from one to 9 per litter and averaging 5.2 per litter. The 7 litters of newborns ranged from one to 6 per litter with an average of but 3.4 per litter. Nine fetuses were found in three litters. These all came from dogs used in the Physiology Department and so they were the usual heterogeneous collection of dogs which one generally finds. They were chiefly mongrels but some were large and some were small. The fetuses were taken from dogs used only in

short physiology experiments so that the experimental work might not affect the growth or condition of the fetuses.

The newborn puppies were born at the animal house some time during the night; brought to the laboratory the first thing in the morning; chloroformed and then treated in the same manner as the fetal dogs. The fetuses ranged in body weight from three to 410 gm and the heaviest newborn puppy weighed 493.5 gm. They ranged from 55 to 257 mm in nose-anus length, measured along the curvature of the back. The newborn puppies were all shorter than the longest fetus.

A 10% solution of formalin was injected into the thoracic and abdominal cavities of each specimen. This was injected with a hypodermic syringe and only enough fluid was injected to comfortably fill the cavities. The top of the skull was opened so that the formalin could enter and preserve the brain and the spinal cord. Then the specimens were immersed in an ample amount of the 10% formalin solution for at least three months. When ready for study, each specimen was removed from the formalin, all excess fluid removed with paper toweling, and then the body weight and body length determined. The body length (nose-anus) was measured with an inelastic string along the curvature of the back. This gave a more accurate measurement than using a stiff metal tape. The larger fetuses and newborns were weighed on a laboratory balance sensitive to 0.1 gm, and the smaller fetuses were weighed on a chemical balance sensitive to 0.1 mg. The smaller fetuses and also all of the organs were weighed in glass stoppered weighing bottles.

As the organs were dissected out they were immediately placed in a moist chamber until transferred to the weighing bottle. The fetuses were also kept in a moist chamber until completely dissected. The fetus was exposed to the air only during the dissection. The other organs have been, or will be reported later.

The pericardium was removed and all vessels were cut close to the heart. The 4 chambers of the heart were opened and all coagulated blood was removed before weighing. The larger

hearts were weighed to the nearest milligram and the smaller hearts to a tenth of a milligram. The lungs and trachea were weighed together in all cases. All vessels and fascia were removed from the lungs before they were weighed in the same manner as the heart. The color of the lungs varied, due to the variable amount of blood included in them. It was impossible to remove the blood from the lungs as was done for the heart and hence there is more variability in the weights of the lungs than in the heart.

The weights and percentage weights will be presented with reference to body weight and to body length (nose-anus). Some of the litters were of known age and it was planned to plot these against age also. When this was tried the variation in the material made these curves less regular than those plotted on body weight or body length. This was undoubtedly due to the great variation in the size of the parents. Some of these fetuses came from large, and some from small dogs, although of course most of them were mongrels. No attempt will be made to use the age as abscissae.

I wish to express my appreciation of the careful work of Dr. R. L. Corder who collected and preserved the first 91 of these specimens; to the members of the Physiology Department; also to the medical students who have saved some of the fetal material for this study.

Heart

The growth of the heart in grams plotted on body weight also in grams is shown in figure 1. The two straight lines starting at 3 gm of body weight, and with the break at 145 gm, represent the growth of the heart. The abscissae represent body weight in grams and the ordinates in the left margin represent both grams and percentages. The dots represent individual fetal male dogs and the dots enclosed by circles, represent newborn males. The circles represent female fetuses and the double circles, female newborn puppies. The

two straight lines were used in fitting these cases rather than a single much more complex curve.

The empirical formulae from which these curves are drawn are:

$$Y = 0.008\,X + 0.031, \text{ from 6 to 145 gm, and}$$
$$Y = 0.006\,X + 0.3226, \text{ from 145 to 440 gm.}$$

In the above formulae, Y represents the weight of the heart in grams and X, the body weight also in grams. All of the linear formulae in this study were fitted by the method of least

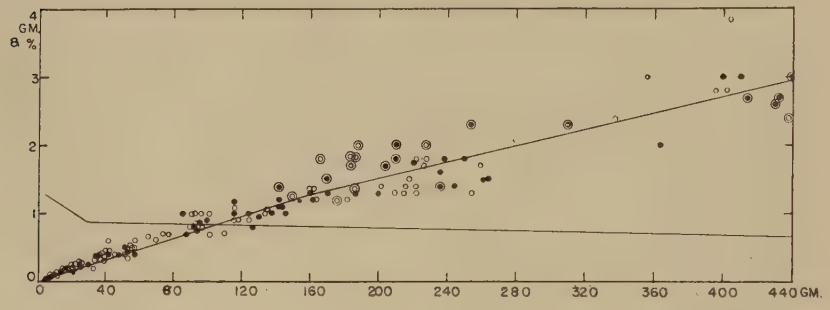

Fig. 1 The weight of the heart in grams plotted on body weight also in grams, is shown in the curve rising from the lower left corner and with the male fetuses shown as dots, female fetuses as circles, newborn males as dots enclosed by a larger circle and the double circles represent newborn females. The lighter line decreasing from its maximum at the left shows the percentage weights of the heart. The empirical formulae from which both curves are drawn are given in the text.

squares (Snedecor, '38). The average percentage deviation of the calculated from the observed values for these two curves is 5.38%.

The lighter line without any cases represents the percentage weight of the heart with reference to total body weight. These individual percentages were plotted on a preliminary graph just as was done for the absolute weights, and then only the lines were drawn on this figure. The scatter of the individual cases was about the same for the percentages as that shown for the absolute weights Again two curves fit these percent-

ages as well as one much more complex curve. These curves were drawn from the formulae:

$$Y = 1.3464 - 0.014\,X\text{, from 6 to 35 gm, and}$$
$$Y = 0.8776 - 0.0005\,X \text{ from 35 to 440 gm.}$$

The average percentage deviation of the calculated from the observed values is 4.59%, or the fit of these curves is slightly better than for the preceding curves of absolute weight.

The same weights of the heart plotted on body length in millimeters are shown in figure 2. The curve is concave superiorly at first and then a straight line. The formula for

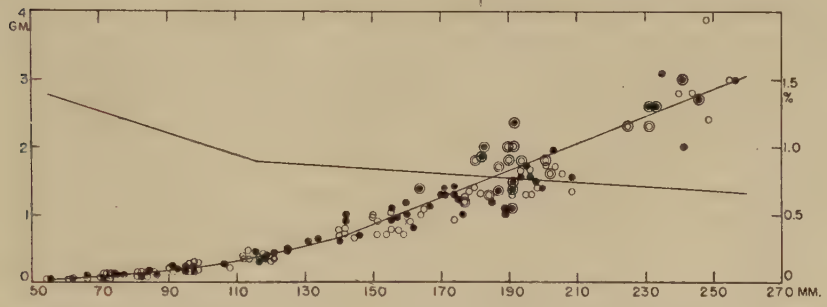

Fig. 2 The weights of the heart plotted against body length. The symbols are the same as in figure 1. The line with the individual cases and rising with the increasing body length represents the increase in absolute weight of the heart. The curve without individual cases and decreasing throughout, represents the weights of the heart as percentages of the body weight. Empirical formulae for both of these curves are given in the text.

the first or concave part of this curve was fitted by trial and error, and the second part, which is a straight line, was fitted by the method of least squares. The individual specimens are indicated as in the first figure. The formulae from which these curves were drawn are:

$$Y = \frac{(0.1\,X)^{2.8}}{2516} \text{ from 60 to 140 mm, and,}$$
$$Y = 0.02\,X - 2.1681\text{, from 140 to 260 mm.}$$

The average percentage deviation of the calculated from the observed values for these formulae is 5.59%.

The line decreasing from the beginning to 116 mm and then more slowly during the remainder of the period represents the heart weight as percentages of the body weight. This curve was drawn in the same manner as the percentage curve in figure 1. The percentage values are given in the right margin. The formulae from which these curves are drawn are:

$$Y = 1.8289 - 0.008\,X\text{, from 60 to 116 mm, and}$$
$$Y = 1.0981 - 0.0017\,X\text{, from 116 to 260 mm.}$$

These curves fit the cases better than any of the other curves, for the average percentage deviation of the calculated from the observed cases is but 3.38%.

Whether the heart weight is plotted on body weight or on body length, the rate of growth slows up in the latter part of the fetal period, and similarly the percentage weight of the heart decreases more rapidly at first and then more slowly. The change in the percentage curve is found later when the percentages are plotted on body length. The use of body length as abscissae tends to space the individual cases over the entire field more than the body weight and this may be a reason for the later change in the percentage curve plotted on body length.

If we take the averages for the first and the last group of specimens (Latimer and Corder, '48), we find that the heart increases from 0.0794 gm to 2.7218 gm or the weight of the heart in the largest group is 34.28 times the weight in the smallest specimens. The percentage weight decreases from 1.26% in the smallest group to 0.678% in the largest, or the initial percentage weight of the heart is 2.16 times that of the largest specimens. During this same period of growth, the total body weight has increased a little over 66 times. Another way of showing these relative changes is that the body weight at the beginning of this period is but 1.51% of the weight in the largest specimens and the heart is 2.92% of the weight in the largest specimens. The percentage weight of the heart is 168.17% of the relative weight in the largest specimens. These are just other ways of showing quantita-

tively that the heart is precocious in its development. The curves of absolute growth and also those of percentage change show that this change takes place more rapidly in the first part of the period and then slows up in the latter part. This is shown also in the slower rate of increase in the absolute growth curves and also in the slower rate of decrease in the percentage curves in the latter part of the period.

The relative weight of the heart in the early fetal period is not well established. Scammon ('33) says that the heart of the human fetus of the second fetal month forms 3.6% of the body weight. Patton ('46) gives absolute weights and body weights from which one can easily find the percentage weight of the human fetal heart decreasing from a maximum of 0.76% in the smallest fetuses. The dog heart with a percentage of 1.26% is lighter than the early fetal cat heart with a percentage of a little over 2% (Latimer, '42). Figures for the relative weight of the human heart at term are more uniform. Patton ('46) gives 0.64%; Scammon ('33), 0.7% and Dragendorff ('38) gives 0.76% for males and 0.80% for females. At term the cat heart forms 0.76% of the body weight and the dog, 0.68%. From these variable data it is difficult to draw conclusions as to the relative position of the dog heart in the early part of the fetal period. More data are needed on the prenatal growth of the heart. The retardation in absolute weight shown for the dog heart is not found in the cat heart (Latimer, '42) although in both forms there is an initial decrease in the percentage curve. In the latter part of the period this line remains horizontal in the cat but it continues to decrease throughout the entire period in the dog (see figs. 1 and 2).

Lungs

The weights of the lungs include the weight of the trachea and bronchi in all cases. All vessels, fat and lymph glands were removed. The vessels were cut close to the lungs. The weights of the lungs are shown plotted on body weight in the single straight line in figure 3. The dots, circles, dots enclosed

by circles and the double circles indicate respectively male fetuses, female fetuses, newborn males and newborn females as in the preceding figures. The numbers in the left margin give the weights in grams and the abscissae, the body weights also in grams. This linear curve was drawn from the formula:

$$Y = 0.03\,X - 0.2048, \text{ from } 15 \text{ to } 440 \text{ gm.}$$

This is the poorest fit of any of the curves in this report. The average deviation of the calculated from the observed values

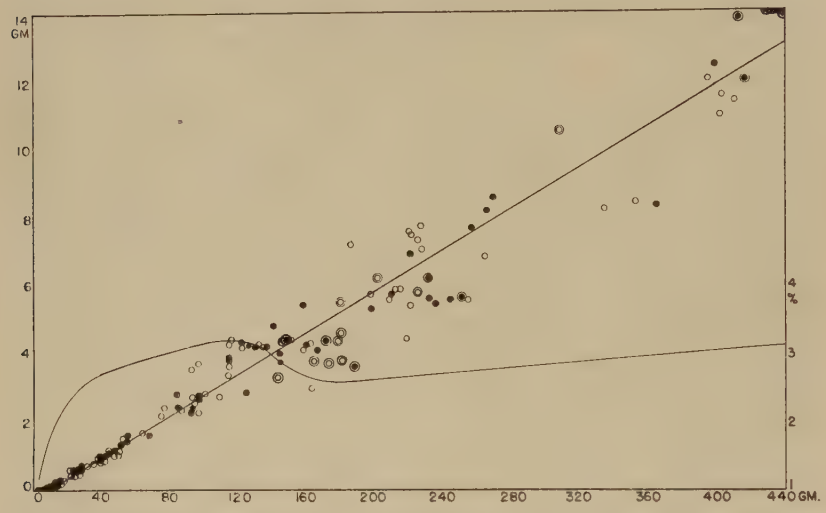

Fig. 3 The weights of the lungs and trachea plotted against body weight are shown in the straight line. The symbols are the same as in figure 1. The empirical formula from which this curve is drawn is given in the text. The percentage changes of the lungs are shown in the irregular line without any cases. No formula is given for this curve. The percentages are shown in the right margin and the weights in grams in the left margin.

is 10.48% This curve, like all of the linear curves, was fitted by the method of least squares. The cases are more scattered than in the other figures. The variability in the color of the lungs has already been mentioned and this was undoubtedly due to the variable amount of blood in the lungs. This blood, of course, could not be removed as was done for the coagulated blood, found likewise in variable amounts in the heart.

The weights of the lungs in terms of percentage of the body weight were plotted as was done for the heart and the irregular line, without any cases, represents the line drawn by inspection through the percentage weights on the preliminary graph. The percentages are given in the right margin. The lungs rise rapidly at first from about 1.4% to a maximum

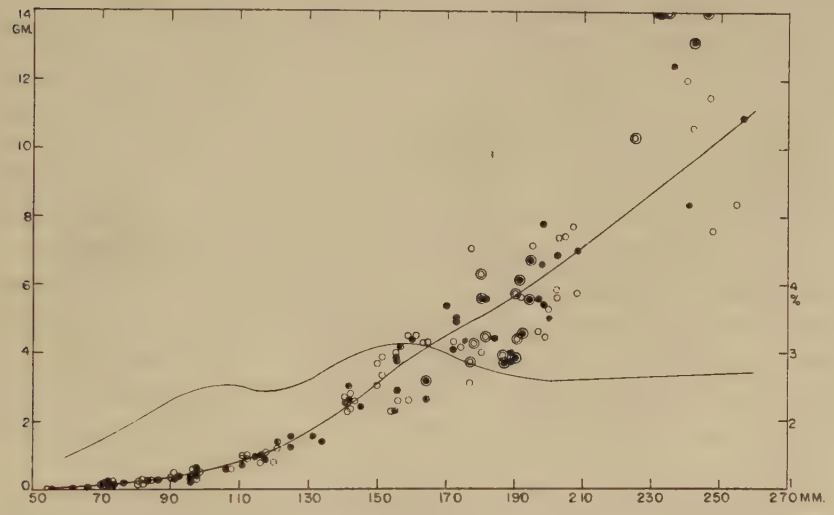

Fig. 4 The weights of the lungs plotted on body length are shown in the curve concave superiorly. The symbols for the individual cases are the same as in figure 1. The formulae for this curve are given in the text. The irregular line without any cases shows the percentage changes in the lung weights. The percentage values are given in the right margin and the weights in grams in the left margin.

of 3.18% at 120 gm of body weight, then follows a rapid decrease and a slow rise to about 3.1% in the largest fetuses. Some of the newborn dogs are shown in the extreme upper right corner of this figure. These evidently had breathed and their lungs were not used in determining this growth curve.

The same weights of lungs, trachea and bronchi, plotted on body length measured in millimeters, are shown in figure 4. These cases are most easily fitted by two curves, both

concave superiorly. They were drawn from the following empirical formulae, determined by trial and error:

$$Y = \frac{(0.1\,X)^{4.3}}{37536} \quad \text{from 60 to 157 mm, and}$$

$$Y = \frac{(0.1\,X)^{2.2}}{115} \quad \text{from 157 to 250 mm.}$$

These curves fit these cases a little better than the curves in figure 3, but they are not as well fitted as the curves in figures 1 and 2. The average deviation of the calculated from the observed values for these curves showing lung weight on body length is 6.99%. The weights of the lungs shown plotted at the top of the figure are those of newborns and they were not used in determining the formulae for the curves.

The lighter irregular line represents the percentage weights of the lungs. This line, like the percentage curve in figure 3, was drawn by inspection. The percentages are given in the right margin. The maximum percentage comes when 61.5% of the body length, or 27.3% of the body weight (fig. 3) has been reached. The use of body length as abscissae tends to scatter the smaller specimens and to more closely group the larger specimens.

During the fetal period the weight of the lungs increases from 0.1027 to 12.3968 gm or an increase of 120.7 times compared with the increase of a little over 34 times for the heart weight. At the beginning of the fetal period the lungs are but 0.8% of their weight at term. The maximum percentage weight of the fetal dog lungs is found at about 120 gm of body weight or 160 mm of body length. At this maximum, the percentage weight of the lungs has increased about 2.5 times.

There are very few data on the prenatal weights of the lungs. Scammon ('33) reports a maximum percentage weight in the human fetus of about 3.3% at the 4th fetal month. He gives the percentage weight at term as 1.7% which is less than that found for the dog lungs. The lungs of the cat (Latimer, '43) reach a maximum of about 3% and then form about 2% at term. Thus the weights and the percentage

weights of the fetal dog lungs follow the same general pattern found in man and in the cat.

The ratio of the lung weight to the heart weight was determined for each of the averages used in determining the formulae (see Latimer and Corder, '48). The ratios increased from 1.36 in the smallest group to about 120 gms of body weight when the lungs were 4.04 times the weight of the heart. Then the ratios dropped to 3.20 and again began to increase more slowly until in the largest specimens the lungs were 4.49 times the weight of the heart. This curve of lung-heart ratio (not shown here) was much like the curve of percentage weight of the lungs shown in figure 3. The increase in the heart is less than that of the lungs throughout the fetal period. The growth of the lungs is more rapid in the earlier part of the period while the heart growth is more uniformly distributed over the entire period. The heart is relatively lighter in the fetus at term than it is in the early fetus, while the lungs are relatively heaviest in the fetus at term, with a second maximum early in the fetal period.

SUMMARY

Empirical formulae are given so that the weight of the heart or the lungs, including the trachea and bronchi, may be determined from either the body weight or the body length.

The heart increases as a linear function of the body weight and as a linear function of the body length in the latter part of the fetal period. In the first part of its growth against body length the curve is concave superiorly. The heart forms its maximum percentage (1.26%) of the body weight at the beginning of the period and this decreases rapidly at first and then more slowly to about 0.68% in the fetuses at term.

The lungs and trachea increase as a linear function of the body weight and they form curves concave superiorly when plotted on body length. The lungs increase irregularly or from a percentage of about 1.4% at first to a

maximum of 3.18% at 120 gm of body weight and then decrease and come back to nearly this percentage at term.

The heart increases 34 times, the body weight 66 times and the weight of the lungs and trachea 121 times during the fetal period.

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NARROWING OF THE SECOND PART OF THE SUBCLAVIAN ARTERY

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ONE FIGURE

During the course of an investigation designed to elucidate the basis of the peripheral vascular complications in the upper limb associated with abnormal ribs and soft tissues relations at the cervico-thoracic junction, it became evident that the lumen of the subclavian artery was normally reduced where the vessel coursed behind the scalenus anterior. Despite the voluminous literature on the anatomy of the subclavian artery and the peripheral circulatory disturbances consequent on compression of the vessel at the root of the neck there is surprisingly little reference to this normal narrowing. A search of the literature has revealed only two previous references to this morphological feature. One is contained in Poirier and Charpy's Traité d'Anatomie Humaine which quotes Stahel's figures for the area of the lumen of the first, second and third parts of the left artery as 27, 15 and 20 mm^2 respectively. In Stahel's opinion the narrowing was in no way connected with the disposition of the scalene muscles in relationship with the artery but rather to be explained as a consequence of the constriction occurring in any moving stream at the apex of a curved tube so that "là où la veine liquide se contracte, le vaisseau devient nécessairement plus étroit." Dubreul ('26) found the narrowing in only "trois ou quatre dissections" and concluded "Dans plusieurs autres cas il était à peine indiqué; la plupart du temps il n'y en avait pas trace."

In view of the possible bearings of this feature on the genesis of peripheral circulatory disturbances the subject was considered worthy of further investigation. This paper is a brief report of the findings of such an enquiry.

MATERIAL AND METHODS

Observations were made on 13 specimens of the subclavian artery from adult formalin-preserved dissecting room subjects and on 22 autopsy specimens while, in three full term foetuses, the vascular tree was injected with a radio-opaque material, X-rayed, and the width of the subclavian artery studied at various levels. The soft tissue relations at the root of the neck were studied in a further 100 dissecting-room specimens. In the adult material, specimens were selected in which the lumen of the vessel was not distorted by arterial disease. In addition, those which gave origin to branches deep and lateral to the scalenus anterior were discarded so that a standard set of material was available for metrical examination. In each specimen the cross-sectional area of the lumen was calculated from diameter measurements taken at the following three levels: at the medial border of the scalenus anterior just distal to the origin of the branches, behind the muscle, and just beyond its lateral border. Transverse and longitudinal sections of the vessel wall at these three levels were obtained for histological examination. These were prepared and cut in the usual manner and stained with haematoxylin and eosin and with Van Gieson.

In this report the term constriction will be used to describe a reduction in the lumen of the artery which has been established as a permanent feature of the vessel; the constriction is not to be regarded as a temopary feature which is dependent on the continuous activity of the factors responsible for its induction though the latter may add to the degree of the constriction present. In this sense the terms constriction and narrowing will be used synonymously.

OBSERVATIONS AND DISCUSSION

The salient features emerging from the enquiry call for only brief comment:

1. Information relating to the range of variation of the area of the lumen in the three parts of the artery in each specimen are contained in table 1 A.

TABLE 1

SPECIMEN	A			B		
	CROSS-SECTIONAL AREA, IN MM², OF THE LUMEN OF EACH PART OF THE SUBCLAVIAN ARTERY			COMPARISON OF THE CROSS-SECTIONAL AREAS OF THE THREE PARTS OF THE ARTERY		
	1st part	2nd part	3rd part	Second part compared with the first	Third part compared with the second	Third part compared with the first
1	35.2	23.6	22.0	— 11.6	— 1.6	— 13.2
2	38.5	17.6	27.5	— 20.9	+ 9.9	— 11.0
3	44.0	25.9	38.5	— 18.1	+ 12.6	— 5.5
4	70.1	27.5	35.7	— 42.6	+ 8.2	— 34.4
5	58.1	39.2	38.6	— 18.9	— 0.6	— 19.5
6	37.7	23.6	25.5	— 14.1	+ 1.9	— 12.2
7	46.7	15.9	25.5	— 30.80	+ 9.6	— 21.2
8	44.7	28.3	30.2	— 16.4	+ 1.9	— 14.5
9	78.3	37.7	40.0	— 40.6	+ 2.3	— 38.3
10	39.2	27.3	37.7	— 11.9	+ 10.4	— 1.5
11	52.0	28.3	33.0	— 23.7	+ 4.7	— 19.0
12	50.2	23.6	27.5	— 26.6	+ 3.9	— 22.7
13	52.0	28.3	33.0	— 23.7	+ 4.7	— 19.0
14	82.0	35.5	49.5	— 46.5	+ 14.0	— 32.5
15	81.3	43.4	55.0	— 37.9	+ 11.6	— 26.3
16	75.4	47.3	56.0	— 28.1	+ 8.7	— 19.4
17	55.9	31.4	39.0	— 24.5	+ 7.6	— 16.9
18	56.6	36.7	33.4	— 19.9	— 3.3	— 23.2
19	95.0	29.4	28.4	— 65.6	— 1.0	— 36.6
20	66.7	34.5	55.9	— 32.2	+ 21.4	— 10.8
21	50.2	22.0	37.7	— 28.2	+ 15.7	— 12.5
22	46.7	42.4	45.9	— 4.3	+ 3.5	— 0.8
23	38.5	27.5	43.9	— 11.0	+ 16.4	+ 5.4
24	37.8	31.4	33.0	— 6.4	+ 1.6	— 4.8
25	22.1	27.4	44.0	+ 5.3	+ 16.6	+ 21.9
26	35.5	37.8	38.5	+ 2.3	+ 0.7	+ 3.0
27	55.0	49.5	44.0	— 5.5	— 5.5	— 11.0
28	52.1	44.6	47.5	— 7.5	+ 2.9	— 4.6
29	44.4	40.0	41.3	— 4.4	+ 1.3	— 3.1
30	39.2	37.6	37.6	— 1.6	0	— 1.6
31	47.5	52.2	47.5	+ 4.7	— 4.7	0
32	33.0	29.5	27.0	— 3.5	— 2.5	— 6.0
33	23.1	15.4	21.1	— 7.7	+ 5.7	— 2.0
34	42.5	33.5	37.8	— 9.0	+ 4.3	— 4.7
35	47.3	31.4	31.4	— 15.9	0	— 15.9
Mean	50.7	32.2	37.4			
Maximal area	95.0	52.2	56.0			
Minimal area	22.1	15.4	21.1			

2. All but three specimens showed a reduction in the area of the lumen at the inner margin of the scalenus anterior.

3. In 11 (85%) and 15 (68%) of the dissecting room and autopsy specimens respectively the bore of the vessel increased when the artery emerged from behind the scalenus anterior though not to its original size except in two of the autopsy specimens in which, however, the cross-sectional area of the second part exceeded that of the first.

Five of the autopsy and two of the dissecting room specimens showed varying degrees of further narrowing at the lateral border of the scalenus anterior while two of the autopsy specimens showed no redilatation.

4. The narrowing was usually confined to that segment of the artery behind the scalenus anterior and, contrary to Stahel's account, did not extend beyond it except in the few cases where there was either no re-expansion or a further constriction of the artery.

5. The difference between the area of the lumen of one part of the artery and that of another provides a measure of the extent of the narrowing observed and the manner in which it varied. Values for the individual specimens are given in table 1 B but, for the whole series, may be conveniently expressed in the table on page 303.

6. The narrowing was visible in foetal material (fig. 1) indicating that the factors responsible for it commence to operate at a very early stage.

7. The great variation in the extent of the narrowing from specimen to specimen suggests that the factor responsible operates to a variable degree while the disposition of the constriction leaves little doubt that the scalenus anterior is in some way connected with it.

8. A study of the soft tissue relations at the root of the neck has confirmed the high incidence of the scalenus pleuralis muscle which ranged in form from a few fibers strengthening Sibson's fascia to a well developed muscle extending between the transverse process of the 7th, and occasionally the 6th, cervical vertebra medially and the first rib laterally.

	NUMBER OF SPECIMENS	AVERAGE DIFFERENCE IN MM^2	GREATEST DIFFERENCE IN MM^2	LOWEST DIFFERENCE IN MM^2
First part exceeding the second	32	20.5	65.6	1.6
Second part exceeding the first	3	4.1	5.3	2.3
Second part exceeding the third	7	2.7	5.5	0.6
Third part exceeding the second	26	7.8	21.4	0.7
Third part equalling the second	2			
First part exceeding the third	31	14.8	38.3	0.8
Third part exceeding the first	3	10.1	21.9	3.0
Third part equalling the first	1			

The muscle was present on the right in 80% of the specimens and on the left in 69%. On the right it was developed in 69% of these specimens and on the left in 68%. The artery passed between this muscle and the scalenus anterior, the former separating it from the lower trunk of the brachial plexus.

In 15% of the specimens the scalenus medius inserted well forward on the rib surface, becoming continuous with the scalenus anterior and thereby effacing, more or less completely, the groove on the rib for the vessel. As a result the vessel was lodged at the apex of a V-shaped muscular furrow

and the antero-posterior constriction of the vessel under these circumstances was very obvious.

The lower trunk of the brachial plexus occupied an inconstant position in relationship to the artery being placed either posterior to it, the trunk then intervening between the artery and the anterior fibres of the scalenus medius, or beneath it.

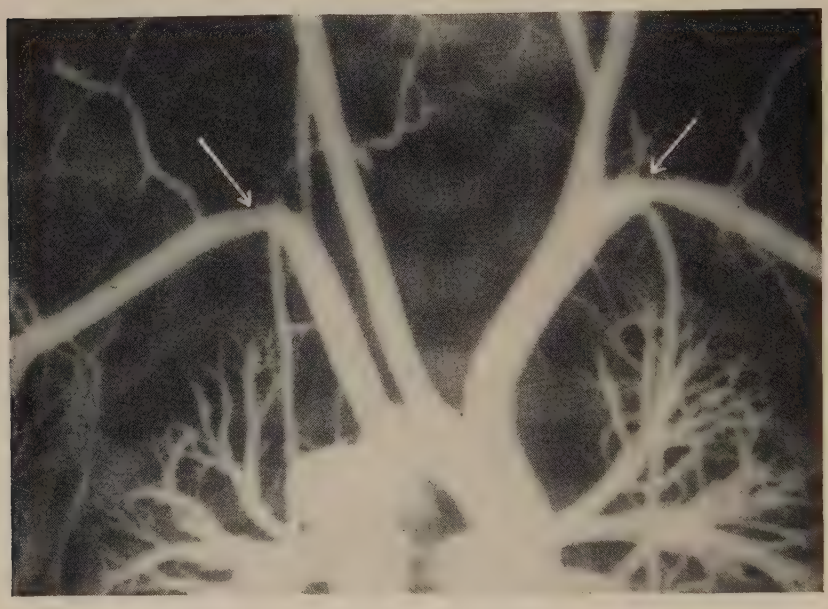

Fig. 1 Injected vascular tree of a full term foetus. The arrows are directed to the subclavian narrowing.

Thus, in the second part of its course the artery is lodged in the interval bounded anteriorly by the scalenus anterior and posteriorly by the scalenus pleuralis medially and the scalenus medius and the lower trunk of the brachial plexus laterally. The dimensions of this muscular interval are influenced by the activity of the muscles defining it but, in the passive state, the interval is usually so narrow that some constriction of the contained artery is inevitable. The subclavian artery is the only major arterial channel passing between two closely applied muscle planes without the pro-

tection of a supporting fibrous or osseo-fibrous ring such as is provided in the case of other channels (e.g., the femoral). Furthermore the arch of the vessel, which represents its second part, is firmly drawn down into this intermuscular space by virtue of its dependent and moveable attachments infero-medially and infero-laterally.

The results of this study indicate that the reduction in the lumen of the arch of the artery arises as the result of pressure exerted by the surrounding muscles as the artery hangs between them. Minor variations in the size, disposition and activity of the surrounding muscles lead to modifications of the forces by whose collective action the vessel is narrowed and this accounts for the observed variations in the extent of the narrowing. It was difficult to establish a correlation between the bulk of the scalenus pleuralis and the degree of the arterial constriction since the dimensions and weights of the muscle were not measured. However, the maximal constrictions appeared to be associated with well developed scalenus pleuralis muscles. In fact the most obvious constrictions were observed in those specimens in which this muscle was so well developed as to give the impresson that the artery was piercing the scalenus anterior which, of course, might well have been the case in some specimens. Compression between the scalenus pleuralis and the scalenus anterior muscles is not, however, the sole factor operating, since in some of the specimens the scalenus pleuralis was absent but the arterial narrowing was present.

The occasional extension of the narrowing to the third part of the artery or an even further constriction is obviously due to factors other than muscular compression though this finding does not exclude this factor from being of etiological significance in producing the narrowing of the second part. Moreover the extent of the further constriction in the few specimens in which it occurred was very small and probably lacks significance.

Of more interest is the dilatation which is customarily found when the artery emerges from behind the scalenus anterior.

Is this dilatation the expression of forces introduced when fluid, flowing in a constricted section of a circuit, is suddenly permitted to expand on emerging from the constricted section? Halsted ('16) examined this proposition experimentally and concluded that constriction must border on the point of almost total occlusion before dilatation occurs in the section distal to the constriction. There is the possibility, however, that this distal dilatation represents, not a true dilatation, but the normal arrangement at that level which has only assumed a dilated appearance on account of the narrowing imposed on the second part of the artery. A comparison of the cross-sectional areas of the various parts of the artery reveals that:

i. In comparison with the first, the second part of the artery was rarely dilated and when it was the extent of the dilatation was insignificant.

ii. In general the greater the narrowing of the second part, as compared with first, the greater the reduction of the lumen of the third part in comparison with the first. This suggests that the extent of the narrowing in the second part may influence the dimensions of the third.

iii. The lumen of the second part rarely equalled or exceeded that of the third; in the case of the latter the difference was so small as to lack significance.

iv. The dimensions of the lumen of the third part were the same as, or greater than, those of the first in only one and three specimens respectively; in the latter the difference was significant in only one specimen.

An analysis of the data in table 1 and the generalisations outlined above support the suggestion, advanced as a result of the present investigation, that the subclavian artery is a gradually tapering channel which has imposed on it a secondary narrowing at the site where it is hung between the scalene muscles. As we have seen, the amount of this secondary narrowing is variable and depends on the development of the factors responsible for it. As a result, the second part may remain larger than the third or may only be reduced to

the same bore, though both these arrangements were unusual. In the great majority of the specimens the constricting force was sufficient to narrow the second part in comparison with the third, and thereby to constrict it, the extent of the narrowing depending on factors already described. Furthermore there is some evidence to suggest that the extent of this narrowing may exert an influence on the size of the lumen of the third part.

9. Histological examination of transverse and longitudinal sections of the wall of each part of the artery failed to reveal any significant differences in microscopic structure.

SUMMARY

Attention is directed to the permanent narrowing of the subclavian artery which normally occurs in the second part of its course where it is hung between the scalene muscles. The extent to which the artery is narrowed at this site varies; the range of variation in 35 specimens is given.

The results of the investigation suggest that the subclavian artery normally undergoes a gradual reduction in size as it passes peripherally and that at the site where it passes between the closely applied scalene muscles, a secondary narrowing is imposed on it by the surrounding structures. Minor variations in the size, disposition and activity of the surrounding muscles lead to modifications of the forces by whose collective action the vessel is narrowed and this accounts for the observed variations in the extent of the narrowing.

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DEMONSTRATION OF FIBERS IN THE DECALCIFIED BONE MATRIX BY ENZYMATIC DIGESTION

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FOUR FIGURES

That the organic matrix of bone contains fibers masked by an amorphous ground substance has been an undisputed fact since the classic work of v. Ebner in 1875. On the other hand, the preparation of histologic slides which consistently display these fibers clearly is difficult even though several special methods of staining have been developed.

v. Ebner observed the fibers in thin, unstained slices of bone which had previously been decalcified in dilute hydrochloric acid containing sodium chloride. According to him the salt minimized the swelling of the collagenous bundles and facilitated their identity. Studnička ('06) was the first to stain bone fibers by Bielschowsky's silver impregnation method. Weidenreich ('23) had great success with his modification of the Gram-Weigert fibrin stain. The results, as illustrated in his chapter in Möllendorff's Handbuch, display the architecture of the bone fibers with amazing clarity. Recently, Ruth ('47) has claimed the unmasking of the fibers by treating bone slabs with potassium hydroxide. His findings indicate that in the Haversian lamellae the fibers are arranged quite contrary to the accepted pattern, as determined by the usual staining methods and with polarized light.

The author, in the course of preparing slides for teaching purposes, has tried the above methods with little success. Bielschowsky's silver impregnation as well as its numerous modifications gave some satisfactory results. However, the

inconsistency of this technique, which is well-known to histologists, diminishes its value either as a routine method or in critical experimental work.

The choice of pancreatin or trypsin in the demonstration of fibers of decalcified bone was purely an empirical one, based on the fact that collagen is relatively resistant to either of these enzymes. This report describes briefly the technical procedures and adds some comments on the results. By the use of this method a current study is being made of bones of several species including man.

METHOD

The use of pancreatin or trypsin in the isolation of collagen from other components of fibrous connective tissue is familiar to both histologists and physiological chemists. It application towards the direct microscopic observation of bone fibers has not, as far as the writer knows, been reported. Except for minor changes, the steps described here closely follow those given in Romeis' Taschenbuch der Mikroskopischen Technik.

Fixation and decalcification. Pieces of bone about 5 mm in thickness are fixed in Carnoy's fluid for 5 hours and then decalcified for about two days by direct immersion in 70% alcohol containing 5% nitric acid. Each piece is sliced into halves to reduce thickness and washed in running tap water for at least 24 hours since complete removal of acids and fixatives is essential. Water is removed in the usual manner by passage through 70, 80, 95 and 100% alcohol.

Removal of fat. The tissue is transferred from absolute alcohol to benzine for 5 days. A daily change of the solvent is preferred. The specimen is then hydrated in the usual manner by passage through decreasing concentrations of alcohol to sterile distilled water.

Digestion. A 0.3% solution of Pancreatin U.S.P. or 0.2% Trypsin (Armour) in sterile distilled water is prepared and alkalinized by adding 0.3 gm of sodium carbonate per 100 cm^3. Sterile Erlenmeyer flasks with cotton plugs are ideal

containers. The amount of bone placed in the digesting solution should be about 50 times the dry weight of the enzyme. The tissue is digested for two days at 37.5°C., washed in several changes of distilled water, dehydrated in alcohol and embedded in paraffin or celloidin. Sections should be cut as thin as possible to obtain a clear picture of the bone fibers.

Staining. Any of the procedures used in demonstrating dense fibrous connective tissue are satisfactory. Staining, in this case, is no longer a critical factor because the section consists almost entirely of a single tissue constituent. For maximum "sharpness" of the fibers, however, Gomori's silver impregnation or Mallory's aniline blue collagen stain is recommended. (Both methods are described in Mallory's Pathological Technique, and in many of the recent texts on histologic procedures.) The impregnation method is rapid, consistent, and has the added advantage of differentiating tinctorially the axes of the fiber bundles.

RESULTS AND COMMENTS

The progressive stages of digestion can be followed in a relatively thick piece of bone digested for a period longer than the one suggested above. Figure 1 shows the outer portion of a transverse segment through the tibial diaphysis of an adult dog. This specimen was digested by incubating at 37.5°C. for 4 days in 0.3% pancreatin, the enzyme solution having been renewed after the second day. In the area of the outer circumferential lamellae (*O*) the finer fibers have been destroyed and only the coarser ones persist. The dark area at the right of the figure represents the center of the compactum and is unaffected as yet by the enzyme. The matrix here appears homogeneous as in ordinary histologic preparations of bone tissue. The intermediate area (*i*) along the center of the figure which contains the Haversian systems and interstitial lamellae shows what is probably the optimum stage of digestion. When stained with Gormori's silver method, fibers cut transversely stain brownish-orange, while those cut longitudinally are either gray or black. Unfor-

tunately the photomicrographs reproduced here fail to exhibit this differentiation. The fiber-free cementing lines, devoid of their amorphous cement substance, are seen as sharp, irregular clefts.

Thin slices of decalcified bone (2 to 3 mm in thickness) incubated in the enzyme solution for two days results in minimum destruction of the fine fibers, as well as distortion of its architectural pattern (figs. 2, 3 and 4). Figure 2 illustrates a cross section through the shaft of a humerus from a human fetus at term. The intercellular stroma of the periosteum (*p*) is well-preserved and strands from it continue as Sharpey's fibers into the bone matrix. The coarse fibrous nature of the latter is unmasked to its fullest extent, for both protoplasmic and amorphous contents of the decalcified bone have been removed by enzymatic action. Figure 3 typifies the complex arrangement of the fibers in the spongiosa of an adult dog bone. There is some derangement of the lamellae along the site of the cementing lines. Careful handling of the specimens prior to embedding minimizes the occurrence of such distortions. Favorable tangential views as in figure 4 of the Haversian systems (*h*) clearly demonstrate the matting of the "longitudinal" fibers of a lamella. Along the periphery of the system, located in the center of the figure, the cut ends of the "transverse" fibers appear as coarse, dense rods and granules.

Both trypsin and pancreatin in their respective concentrations gave equal results. The writer has been unable to detect any significant advantage of one over the other. While the method admittedly is time-consuming, critical steps are not encountered. It is felt that the easy and consistent attainment of good histologic slides justifies the introduction of this technique.

The author acknowledges the encouragement and aid given him by Dr. Otto F. Kampmeier, Chairman, Department of Anatomy, University of Illinois College of Medicine, Chicago, Illinois.

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PLATE 1

EXPLANATION OF FIGURES

Figures 1–4, inclusive, are unretouched photomicrographs of sections stained with Gormori's silver impregnation method.

1 Area of a transverse section through the tibial diaphysis of an adult dog; 0.3% pancreatin digestion, 4 days. × 95. *o*, site of outer circumferential lamellae showing only coarse fibers; *i*, intermediate zone in optimum stage of digestion. The dark area at the right represents the center of the compactum which is not attacked by the enzyme. The inner half of the cortex is not shown.

2 Area of a transverse section through the humeral diaphysis of a human fetus at term, 0.3% pancreatin digestion, two days. × 185. *p*, periosteum; *h*, primitive Haversian canal. Note the cut ends of fiber bundles in the matrix, also the small clear spaces which represent the empty lacunae.

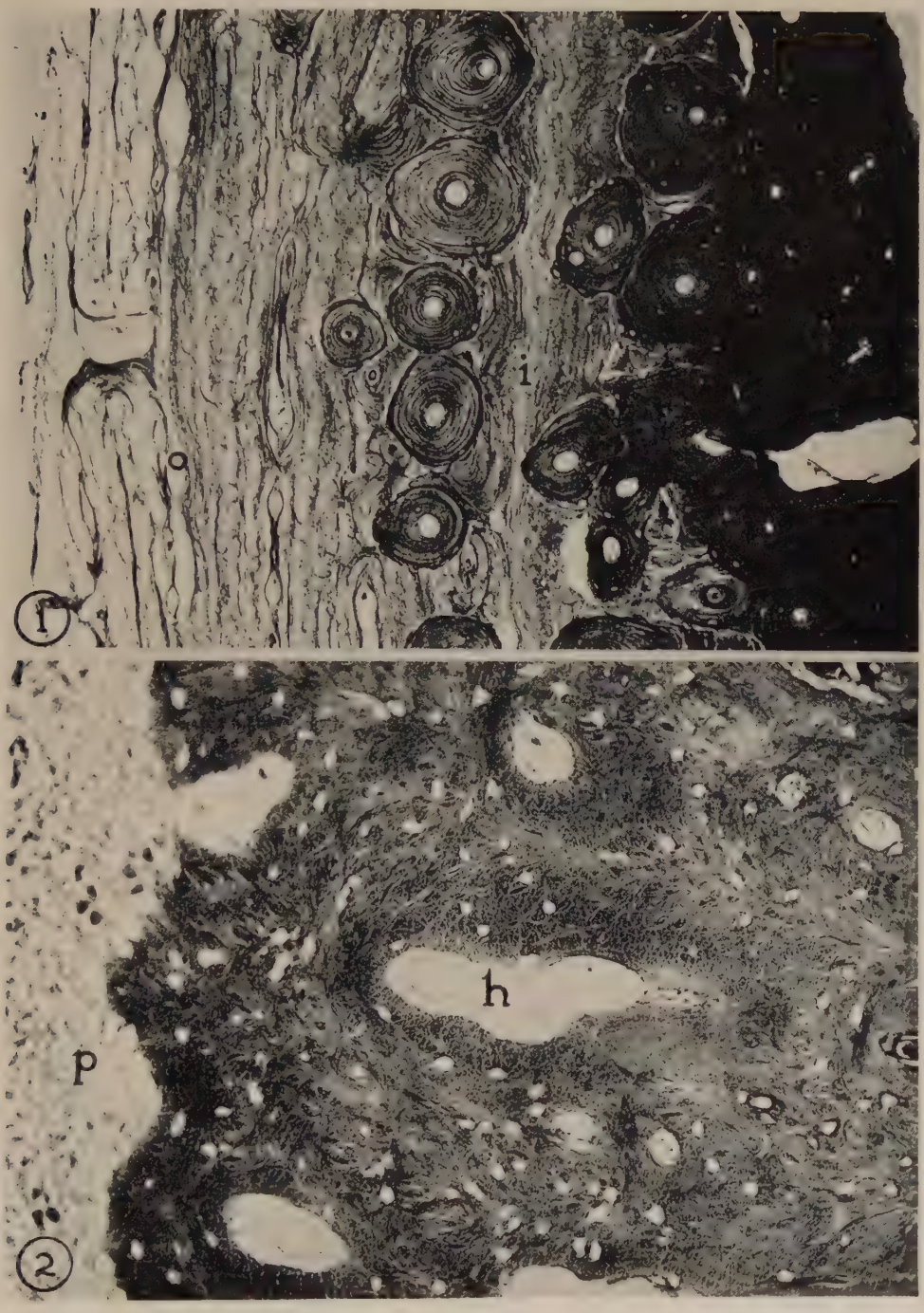
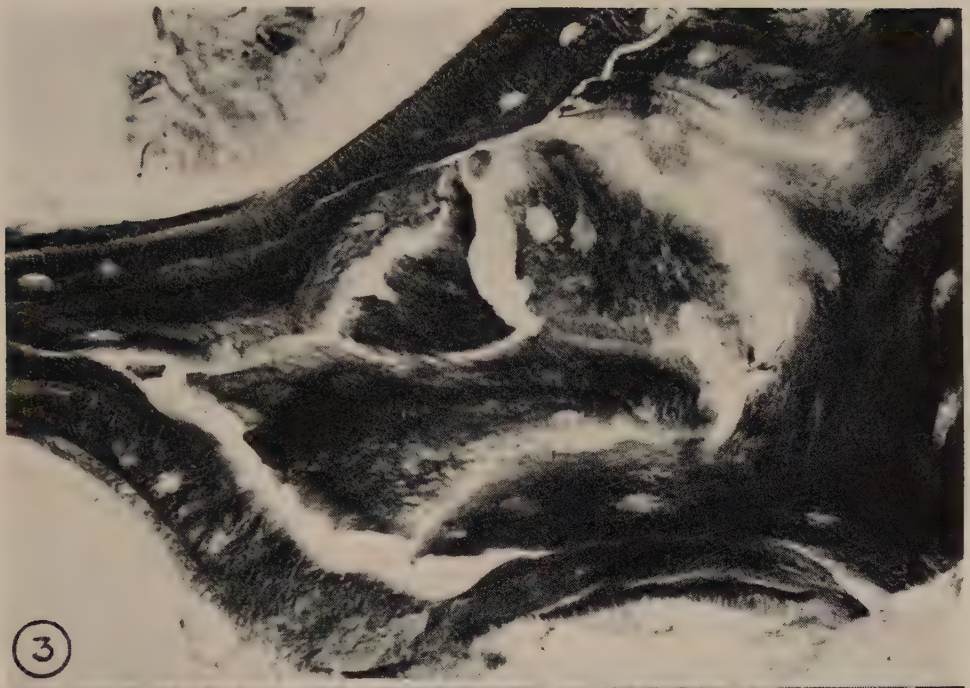

i
o
1
h
p
2

PLATE 2

EXPLANATION OF FIGURES

3 Area of a longitudinal section through the spongiosum of a tibia from an adult dog; 0.3% pancreatin digestion, two days. × 200. The bone spicule joins the compactum at the right. Note partial cleavage and distortion of the lamellae along the irregular cementing lines (empty clefts).

4 Area of a longitudinal section through the femoral diaphysis of an adult dog; 0.2% trypsin digestion, two days. × 185. *h*. Haversian canal. The tangential view of the Haversian systems show the weaving of the ''longitudinal'' fibers of a lamella. Note the cut ends of the ''transverse'' fibers which appear as dark rods and granules.



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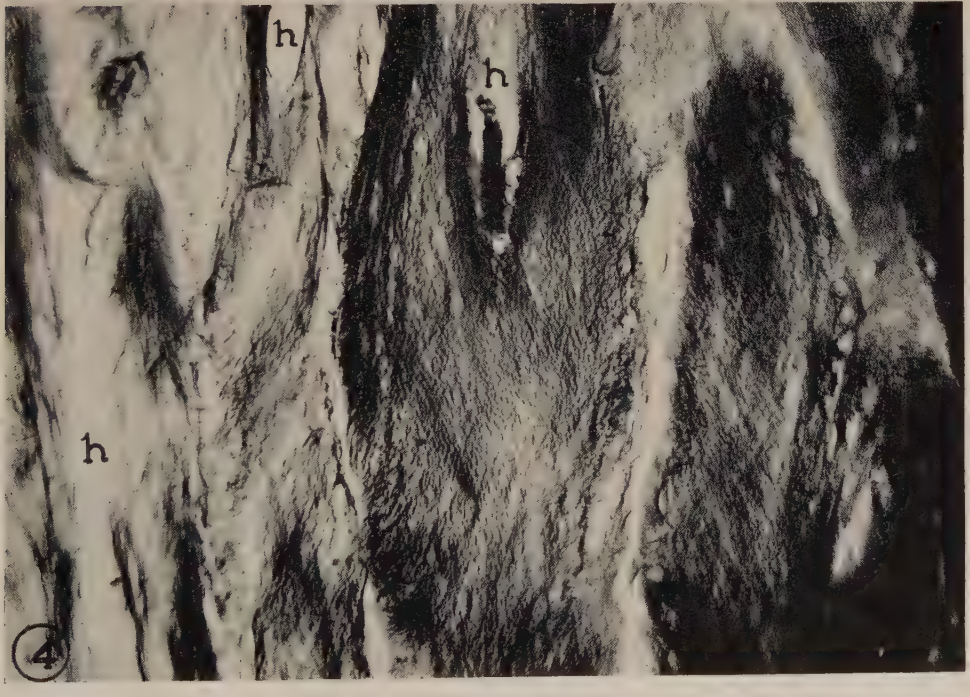

4

A METHOD FOR RADIOAUTOGRAPHY OF SPECIMENS COMPOSED OF BOTH HARD AND SOFT STRUCTURES

REIDAR F. SOGNNAES, JAMES H. SHAW, ARTHUR K. SOLOMON AND EGIL HARVOLD[1]

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SEVENTEEN FIGURES

The preparation of radioautographs of bones, teeth and jaws is complicated by the close proximity of such different types of structures as: the soft bone marrow, periosteum, tooth pulp and periodontal membrane; the semi-soft cartilage, immature enamel and dentin; and the extremely hard bone and mature enamel and dentin. The relative radioactivity of these various structures in their normal composition and relationship is almost impossible to record in a radioautograph through the use of sections prepared by routine methods.

Acid solution of the inorganic salts during the preparation of decalcified sections in the first place removes from all calcified tissues those radioactive isotopes usually used in hard tissue investigations, such as calcium, phosphorus, fluorine, etc. Secondly, uncontrolled decalcification procedures cause the complete loss of the organic framework of the enamel. Ground sections prepared by commonly used methods are too thick or, if made sufficiently thin, are too fragile for the preparation of satisfactory radioautographs. In addition, in routinely prepared ground sections, only the fully calcified regions are satisfactorily preserved, while the semi-soft, immature

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hard structures, as well as the soft structures, are distorted or completely lost. Up until the present time, investigators have had to resort to a prolonged procedure of infiltration and hardening in celloidin in an attempt to secure sections of undecalcified bone (Axelrod, '47). As pointed out in a review of this subject by Axelrod and Hamilton ('48), the time-consuming celloidin technique, which is the only procedure previously described for radioautography of undecalcified bone, is not practical in studies with isotopes that have short half-lives.

The radoautography technique to be described in this paper utilizes a recently developed plastic petrification method (Sognnaes, '47), which, in the period of a few days, permits the preparation of a series of thin ground sections of the combined soft, semi-soft and fully calcified structures of teeth and bones in their undisturbed composition and relationship. The application of the method is exemplified by radioautographs of bone, enamel, dentin and supporting structures of the teeth from monkeys previously injected with radioactive phosphorus (P^{32}).

PROCEDURES

Where the time factor is of minor importance, that is, in tissues having high concentrations of a radioactive material, or in tissues containing an isotope with a relatively long half-life (over two weeks), the original plastic petrification technique can be applied directly, as described in detail elsewhere, (Sognnaes, '47). This paper presents a brief step-wise description of a modification of the above technique which, by reason of its rapidity, has proved useful in the study of tissues which contain an isotope with a relatively short half-life, or which exhibit a relatively low radioactivity, or both. Conventional fixation and dehydration procedures have been dispensed with in this technique, in which each step is reduced to the minimum amount of time required for fresh, single human or animal teeth, or the bones of small animals. Certain increases in these times will be necessary for larger

specimens, especially where it is desirable to retain large amounts of soft tissue in addition to the hard structures.

1. *Fixation and dehydration* by one or more changes of dioxan for a total minimum of 5 hours.

2. *Infiltration* with inhibitor-free methyl methacrylate monomer [2] for a minimum of two hours.

3. *Embedding* in a heavy syrup consisting of methyl methacrylate partly polymerized by the addition of a catalyst.[3] Small, expendable glass shell vials, 0.5–1.0 inch in diameter were found to be suitable for the embedding of teeth or small bones. Chips of polymerized methyl methacrylate may be placed at the bottom of the vials to prevent the teeth or bones from touching the glass and thus being incompletely surrounded by the plastic. In order to allow rapid polymerization, it is advisable to have as low a column of plastic as necessary to cover the specimen.

4. *Polymerization* is produced by intermittent heating of the vials containing the syrup and teeth in an oven at about 50°C. for a period of about 15 minutes, followed by 30 minutes at room temperature. This process should be continued until polymerization is complete. The vials must be kept closed by corks throughout this step to reduce evaporation. If the syrup is successfully prepared, it is possible to complete the polymerization of a one-half inch tall column of the embedding syrup in about three hours. The vial may have to be broken to remove the plastic block.

5. *Cutting.* The specimen, now infiltrated and surrounded by a block of hard plastic, can be cut into slabs either with a fret saw, or with parallel, circular steel saws, as described

[2] The inhibitor-free monomer was prepared by removing the inhibitor (hydroquinone) present in commercial methyl methacrylate monomer by washing with 5% aqueous solution of potassium hydroxide in a separatory funnel. After the monomer was rinsed thoroughly with water and the surplus water drained off, the monomer was dehydrated with anhydrous cupric sulphate and filtered.

[3] The catalyst was made by dissolving 5 gm of benzoyl peroxide in 100 cm^3 of inhibitor-free monomer. One part of this solution was then added to 10 parts of inhibitor-free monomer. The resulting mixture was carefully heated to 70°C., intermittently cooled in ice water, heated and cooled repeatedly until the consistency of a thick syrup had been obtained.

previously (Sognnaes, '47). By the latter means, a tooth or bone can be cut into 5 to 10 thin slabs in one operation.

6. Grinding. The slabs can be ground directly by hand against grinding wheels. If very thin sections are desired, the slabs can be mounted, previous to final grinding, on methyl methacrylate microscope slides with plastic cement (Sognnaes, '47).

7. Autography. Radioautographs of the finished sections can be made by placing them on photographic film under pressure so that the section is firmly in contact with the emulsion. X-ray cassettes and printing frames have been found adequate for the plastic slides. The period of exposure

TABLE 1

The relative speed of 4 types of film tested in radioautography

TYPE OF FILM	MANUFACTURER'S SPEED INDEX		EXPOSURE TO P^{32} SECTION [1]	
	Daylight	Tungsten	Actual time	Index
			hours	
Kodak Tri X pan	200	160	⅛	144
Kodak Super XX	100	80	4	12
Kodak M-plate	32	20	24	2
Kodak Micro-file	..	3	48	1

[1] Radioautographs developed in Kodak DK 20.

must be determined by the sensitivity of the film and the radioactivity of the sample. The latter variable depends upon the type of isotope and its distribution (Steinberg and Solomon, '49), the type of tissue and the thickness of the section. Rough determination of the total radioactivity of a section in a Geiger dry counter was found to serve as a useful guide in the appraisal of the required exposure time.

The speed of the films indicated by the manufacturers for the spectrum from daylight, or from a tungsten light source, did not necessarily correspond to the speed obtained when the film was exposed by the emanation from radioisotopes. In the case of radioactive phosphorus, the speed ratio of 4 different film types tested is shown in table 1. It seemed that films which were particularly sensitive to red

were among the fastest for radioautography of tissues emitting the soft beta rays of P^{32} radiation. This may be due to the size of the grain, the thickness of the emulsion, or special dyes present in red-sensitive emulsions. More work is needed on this phase of the problem, and is in progress (Steinberg and Solomon, '49).

8. Selective autography. Radioautographs of areas with low radioactivity (enamel of erupted teeth) can be prepared independently after the complete removal of the more highly radioactive tissues, dentin, cementum, etc., from the plastic-mounted sections by dental drills (S. S. White Inverted Cone no. 33¼ and Plug Finishing Burs no. 242). The freedom of the remaining enamel from dentin could be checked and the last traces of highly radioactive tissues removed while the section was being observed through the microscope. The section itself and the plastic slide on which it was mounted could be cleaned with a brush sufficiently well that none of the radioactive particles made in this process remained on the slide to interfere with the preparation of the radioautograph. In the terms of total time expanded, Steps 1–4 (embedding) require a minimum of 10 hours, steps 5 and 6 (cutting and grinding) a minimum of one hour, and steps 7 and 8 (exposure) from a few minutes to a month or more, depending upon the sensitivity of the emulsion and the radioactivity of the sample to be autographed.

9. Mounting of permanent slides. The polymerized methyl methacrylate may be removed by immersing the section in the inhibitor-free monomer.[4] The section may be transferred to xylol, stained, and mounted on a glass slide with balsam. In this way, a permanent preparation with better histological detail can be made available for microscopic examination.

The accompanying illustrations of the technique are from various specimens of rhesus monkeys sacrificed two days (monkey A), and 9 days (monkey B), respectively, following intravenous injection to each of 15 millicuries of P^{32}. Where the tissues studied have exhibited an evenly distributed radia-

[4] See footnote 2.

tion, it will be noted that the autographs have very sharp edges with little or no diffusion. It is believed that the plastic material infiltrating and surrounding the edges of the sections has a favorable screening effect on lateral spread of radiation. Another favorable feature of the plastic embedding method is the fact that no coverslips separate the sections from the film during exposure. The same applies during the subsequent microscopical examination of the specimen and its radioautograph if one desires to superimpose one upon the other.

SUMMARY

A radioautography technique is described which utilizes a recently developed plastic petrification method (Sognnaes, '47) that permits the preparations of a series of thin ground sections of combined soft, semi-soft and fully calcified structures in their undisturbed composition and relationship. Jaws and teeth in various stages of development, which exemplify such structural combinations, have been used to illustrate the type of information obtainable through the use of this technique.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Figures 1–17 Radioautographs of plastic-embedded ground sections of various specimens prepared from rhesus monkeys A and B sacrificed two and 9 days, respectively, after the intravenous injection of 15 millicuries of radioactive phosphorus (P^{32}). Where counts of radioactivity are presented, they represent relative values obtained by placing the slide on the second shelf from the window of the Geiger-Müller tube. (A more detailed discussion of the information demonstrated by the following illustrations will be presented elsewhere along with measurements of the P^{32} turnover recorded on a unit weight basis for representative areas.)

1 Sagittal section through mandible of monkey A indicating the relative radioactivity of alveolar bone, of predentin, dentin and enamel of unerupted first premolar and of the erupted, partly resorbed deciduous first molar. Rough dry count of whole section gave 5,710 counts per minute (4 minutes exposure, Kodak tri X film). × 4½.

2 Longitudinal section through unerupted canine from monkey B. Extremely high concentration of radio-phosphate in root end (7,040 c/min.), predentin zone and surface layer of the enamel (24 hours exposure on Kodak M-plate). × 4.

3 Cross section of mandible in the region of the unerupted third molar from monkey A. The radioactivity of the calcifying enamel and the bone is very marked as compared to the dentin (11,520 c/min.; 23 hours exposure on Kodak M-plate). × 4.

4 Longitudinal section of unerupted maxillary third molar from monkey A. The external dentin is negative, while predentin and the maturing enamel are very radioactive (whole section 2,400 c/min.; 23 hours exposure on Kodak M-plate). × 6.

5 and 6 Longitudinal sections of developing maxillary third molar removed from the jaw of monkey A. Same animal and same exposure as figure 3. The enamel has taken up the isotope over a wide area. The cervical and external enamel and predentin are particularly radioactive. The dentin has been removed from the serial section shown in figure 6 (whole section 5,120 c/min.; enamel section 1200 c/min.). × 5.

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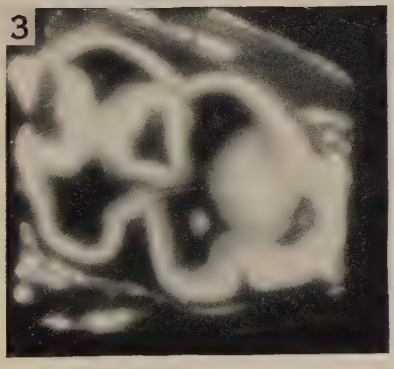
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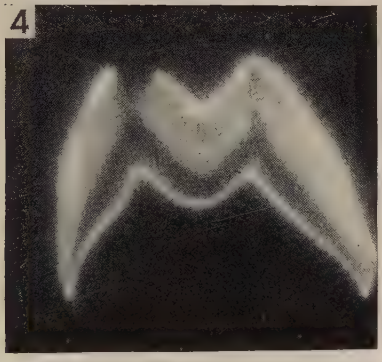
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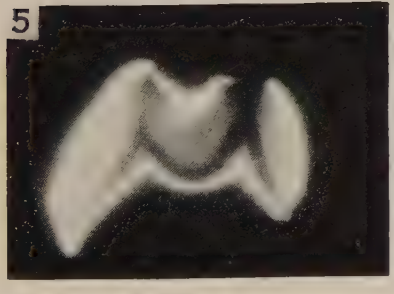
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PLATE 2

EXPLANATION OF FIGURES

7 Unerupted first premolar from monkey B. Cervical region, predentin and surface enamel have a very high radioactivity (total count 4,250 c/min.; exposure 28 hours, Kodak M-plate). × 3.

8 Partly erupted second maxillary molar from monkey B. Surface enamel and predentin very active (1,594 c/min.; exposure 28 hours, Kodak M-plate). × 4.

9 Erupted mandibular incisor of monkey B (836 c/min.; 15 hours exposure on Kodak Super XX, microdol development for 15 min.). × 5.

10 Partly erupted maxillary second molar from monkey B (same as fig. 8 without dentin) (326 c/min.; 15 hours exposure on Kodak Super XX and developed in microdol for 15 minutes). × 2½.

11 Section of erupted incisor (monkey B) from which the dentin has been removed by dental drills. The lingual enamel which is an extremely thin plate in the monkey, shows as one thin line. The labial enamel shows two distinct areas of radioactivity, namely, the internal layer and the surface layer, both most marked in the cervical, less mature portion (exposure 28 hours Kodak M-plate). × 2.

12 Partly resorbed deciduous molar from monkey B (141 c/m.; 15 hours exposure on Kodak Super XX, followed by microdol development for 15 min.). × 3.

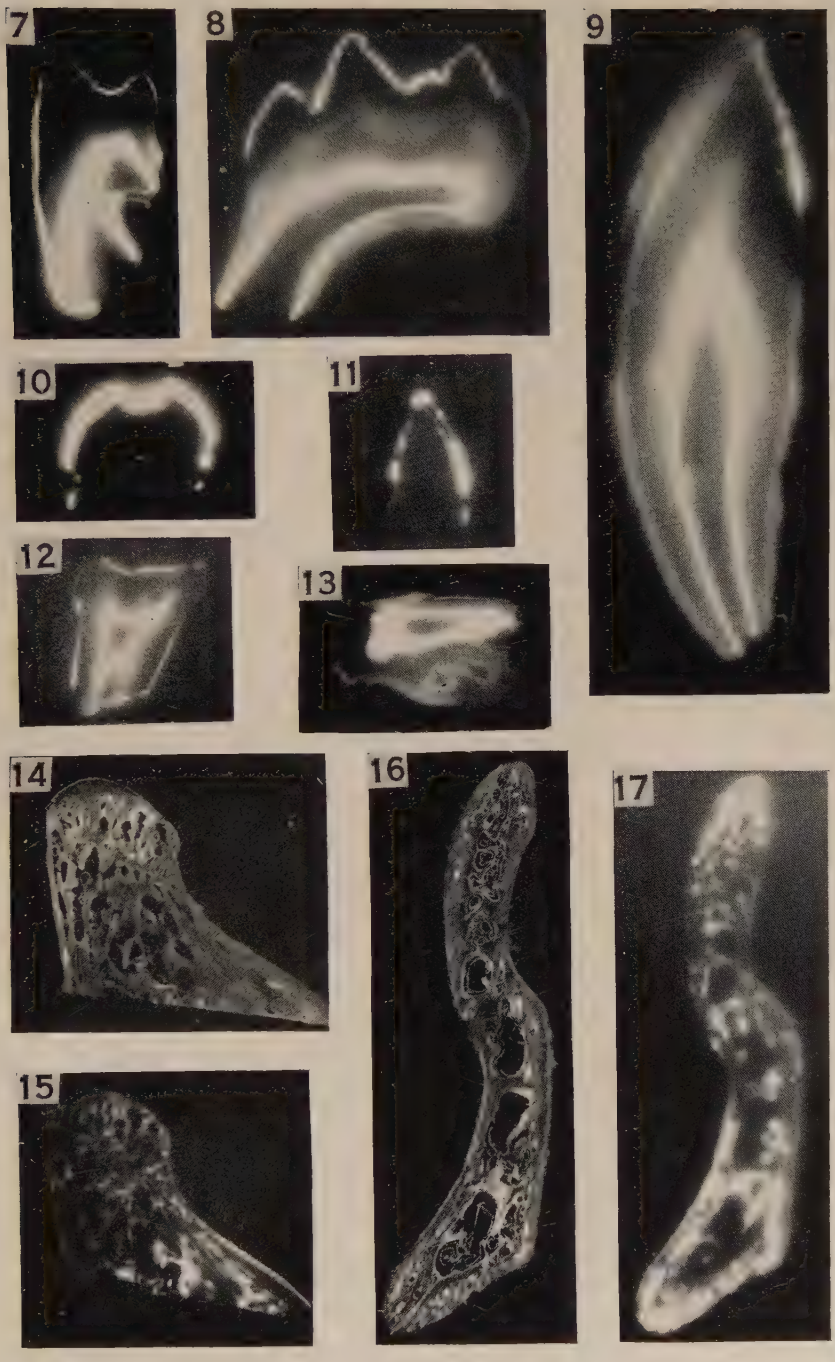
13 Radioautograph of a thumb of monkey B, an example of a non-dental application of this technique to ground sections of structures combining hard and soft tissues (1,800 c/min.). × 2.

14 Parasagittal section of head of condyle with cartilage removed, monkey B. Appearance recorded by light photography. × 3½.

15 Radioautograph of section in figure 14. × 3½

16 Cross section of ramus of mandible of monkey B. Appearance recorded by light photography. × 3½.

17 Radioautograph of section in figure 16. Note activity in the anterior and, more particularly, in the posterior border. × 3½.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EMBRYO-ENDOMETRIAL INTERRELATIONSHIP IN THE RAT AND GUINEA PIG[1]

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TWENTY-ONE FIGURES

Current concepts of the mechanism of implantation include two opposing views as to the specific role of the ovum in the embedding process. Some consider the blastocyst as a parasite-like invader of the endometrium which actively cytolyzes and phagocytizes the endometrial tissue. To this group belong many of the descriptive embryologists who have observed the histologic and cytologic changes at the time of implantation (Spee, '01; Lee, '03; Sansom and Hill, '31; Hill, '32; Bryce, '37; Wislocki and Streeter, '38; Heuser and Streeter, '41; Wimsatt, '44; Hertig and Rock, '45; Alden, '48).

Other investigators have considered the ovum as a more or less inert object and believe that the reaction of the endometrium at the time of implantation is elicited by physical forces such as either localized pressure or trauma, due to the expansion of the ovum, or the loss of the normal surface relationship of the uterine epithelium due to the presence of the ovum on that epithilium (Nicholas, '47). The experimentally induced deciduomata in the rat (Selye and McKeown, '35; Krehbiel, '37) and monkey (Hisaw, '35) have imitated the decidual reactions attendant upon nidation in such detail that the investigators just mentioned maintain that this response is not dependent upon a specific cytolytic action of the

[1] This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

ovum. Additional support for this conclusion comes from the studies of Mossman ('37) on the early stages of implantation in Citellus tridecemlineatus, Tamias striatus and other members of the squirrel family. In these animals abembryonic trophoblastic "attachment cones" penetrate between the uterine epithelial cells. The "epithelial degeneration does not occur at the moment of attachment but does occur somewhat later, always after thickening or edema, whereupon congestion of the subepithelial tissue becomes apparent." Mossman concludes from his own observations on the mechanism of implantation in the Sciuroidea and the reports of others on the artificially induced deciduomata, that: "the stimulus derived from the embryo is not specific" and "seems to be of the nature of a localized irritational one."

Bryce ('37) maintains that "both maternal and embryonic tissues play a part in the process of implantation and therefore there are two sets of phenomena to be analyzed."

The problem of embryo-endometrial interrelationship becomes more complex as investigations bring to light the varying ways in which the trophoblast and endometrium react at the time of implantation in different animals.

In dealing with the specific question of the response of the endometrium to the blastocyst during nidation, we were interested in determining whether inert objects of the approximate size and specific gravity of ova could induce the decidual reaction and indeed implant in the rat and guinea pig. Furthermore, since it has been repeatedly suggested that the implanting ovum may utilize proteolytic enzymes to effect the initial destruction of the uterine epithelium and subepithelial tissues, it was considered important to investigate whether or not such enzymes could be demonstrated in vitro.

EXPERIMENTAL PROCEDURE

Eighty-one female guinea pigs and 68 female albino rats were used in this study. All animals were sexually mature and were maintained under optimal conditions of feeding and housing.

The events in the female reproductive cycle in both groups were timed by the animals' willingness to mate, as determined either by placing them with vigorous males at hourly intervals or by their response to manual manipulation as described by Blandau, Boling and Young ('41) and Young, Dempsey and Myers ('35).

The investigation may be divided into two major parts. The first part consisted of an effort to determine whether inert objects of the approximate size and shape of the blastocyst could induce the decidual reaction and implant in a manner similar to that of the normal ovum. It was not possible to manufacture inert objects which had the same specific gravity as the living blastocyst, therefore, glass and paraffin beads were prepared whose specific gravities were respectively higher and lower than that of the blastocyst. The glass beads were made in the following manner: finely ground glass was thoroughly mixed with cobalt oxide and placed in the glass container "A" (fig. 1). Oxygen, forced into this container through a fine pipette, was directed against the glass-cobalt dust forming a cloud of this material. By means of a Y-tube a gas-oxygen mixture was obtained which, when ignited, produced a flame of high temperature. The glass-cobalt particles, forced into the flame via tube "B," fused into perfectly round, blue or black, glass beads which were collected in a dish of water "C." The diameters of the beads ranged from 10 μ to 175 μ. The size of the beads could be varied somewhat by altering the dimensions of the tip of the Y-tube.

Paraffin beads were prepared by saturating 60°–62°C. paraffin with lamp black in flask "D" (fig. 1). The paraffin mixture was heated to 100°C. By use of the pressure bulb "E" the hot paraffin was forced through the capillary pipette "F" which also was kept hot by a gas flame. The finely divided paraffin mixture was sprayed, either onto the surface of cold water or onto cold glass plates. Perfectly round and smooth, black beads, ranging in diameter from 5 μ to 200 μ were obtained. Glass and paraffin beads of the approximate size of

ova (fig. 2) were selected and stored in separate petri dishes. The glass beads were sterilized before use by boiling in distilled water. The paraffin beads were repeatedly washed in 70% alcohol and rinsed in sterile distilled water.

Ordinarily 4 to 8 beads were taken up into a capillary pipette in a small quantity of Ringer's solution and in the case of the rat, inserted (1) directly into the periovarial space through the thin wall of the periovarial sac, during the period

Fig. 1 Apparatus used for preparing glass and paraffin beads. (A) Glass jar containing ground glass-cobalt oxide mixture. Oxygen was forced into the jar under pressure carrying the glass-cobalt particles via tube (B) into the gas-oxygen flame where fusion of the particles occurred. The perfectly smooth, round glass beads were collected in a dish of water (C). Paraffin beads were prepared by saturating 60°–62°C. paraffin with lamp black in flask (D) and heating it to 100°C. The hot paraffin was forced through the capillary pipette (F) by the use of the pressure bulb (E). The paraffin spray was collected on cold water or cold glass plates.

of heat; (2) into the periovarial space through the peritoneal opening of the bursa, during heat; (3) directly into one oviduct in the region of the dilated ampulla, within 24 or 36 hours after ovulation; and (4) directly into the lumen of a cornu, by piercing the uterine wall in the region of the utero-tubal junction, at either 48, 72 or 96 hours after ovulation. All rats were made pseudopregnant by electrical stimulation of their cervices during heat. The rats were killed for examination from the 5th through the 8th day after ovulation. The cornua were ordinarily cut open along their mesometrial borders and their contents examined under a binocular dissecting microscope. If deciduomata were present they were either carefully dissected to determine the location of the beads, or were fixed in Bouin's fluid and prepared in the usual manner for histologic examination. Ordinarily beads were inserted into one side of the reproductive tract, the remaining cornu serving as the control.

In the guinea pig, beads were inserted either directly into the fimbriated ostium of one oviduct on the day following ovulation, or directly into the lumen of one cornu in the region of the utero-tubal junction, two to 5 days after ovulation. All of the guinea pigs were killed between the 8th and 10th post-ovulatory day and their cornua examined in a manner similar to that described for the rat.

The second part of the investigation consisted of a variety of experimental procedures designed to determine whether or not the implanting ovum secreted a proteolytic enzyme whose activity could be demonstrated in vitro. Guinea pig ova recovered from the 6th through the 8th day, and rat ova from the 5th through the 6th day, after fertilization were placed on various protein substrates including gelatin, purified human fibrinogen and dried homologous plasma. Films of 5 μ to 10 μ in thickness were prepared by dipping or flooding chemically clean glass strips with solutions of these proteins and allowing them to dry. The ova to be tested were removed from the cornua under sterile conditions, placed on a protein film in a small drop of one of the following nutrient

media: Tyrode's solution, Locke's solution, homologous serum, autoplasma with and without embryonic juice, and mixtures of autoplasma, embryonic juice and Simms' ultrafiltrate of beef serum (Simms and Sanders, '42).

The ova cultured on gelatin films were incubated in a moist chamber at 25°C., which is just below the critical temperature for solution of the gelatin (Pickford and Davis, '34). Those cultured on fibrin and plasma films were incubated at 37°C. Incubations were carried out for either 4, 6, or 10 hours. These times were chosen because it had been previously demonstrated that rat ova may withstand culturing for 6 hours at 37.5°C. and 12 hours at 24°C. without signs of histolysis (Nicholas, '34). After the glass strips were removed from the incubator they were gently rinsed in distilled water and stained, either in 1% solution of acid fuchsin or in Delafield's hematoxylin. The strips were then rapidly dried with a fan. Control materials consisted of a drop of one of the culture fluids alone.

The original method of Gates ('30), based on the reduction in density of pieces of exposed photographic film by the release of silver through the progressive proteolysis of the gelatin layers, was thoroughly tested but found ineffective due possibly to the excessive thickness of the film.

OBSERVATIONS

Endometrial reactions in the rat

Normal responses. The general sequence of events during implantation in the rat has been adequately described by Huber ('15), Krehbiel ('37), Alden ('48), and others. However, the examination of a large number of fresh and fixed ova during the earliest stages of implantation have served to clarify certain phenomena which have heretofore not been emphasized adequately. The impression obtained from the literature concerning the problem of the initial contact of the ovum with the uterine epithelium is that after entering the cornua the blastocysts come to rest in certain of the

deeper, preformed crypts along the antimesometrial border, and that the decidual reactions are first initiated within these crypts. This concept originated from the investigations of Nusbaum, (1890) and Burckhard ('01) on the early implantation stages in the mouse and has been generally accepted as describing the condition in the rat (Huber, '15). The preceding description, however, does not conform to the observations we have made on carefully opened, fresh, pregnant cornua, which have not been allowed to contract tonically, as they so often do, after being exposed to the air. These fresh preparations reveal that even though the uterine surface may be somewhat irregular in contour, the ova which have just begun to initiate the decidual reactions do not lie in preformed crypts but have taken up positions in which their abembryonal poles are directed against the rather smooth epithelial surface in the antimesometrial area. The very earliest decidual reactions may be easily discerned by an obvious change in the appearance of the endometrium, in the region of contact, from a translucent greyish color to a more whitish one.

Implantation in the rat is accomplished by two entirely different processes. First, the abembryonal pole of the blastocyst establishes a very tenuous and superficial contact with the uterine epithelium thereby initiating the decidual reaction. The formation of the decidual crypt results from the rapid and eccentric growth of the decidual tissue. In the second stage, which begins some hours after the decidual crypt has been well established, the primary trophoblast giant cells actively cytolyze and phagocytize the uterine epithelium thereby completing interstitial implantation (Alden, '48).

One of the earliest decidual reactions we have observed is that shown in figure 6, representing a longitudinal section through the antimesometrial area of a rat's uterus removed early on the 5th day after fertilization. As yet there is no detectable alteration in the superficial epithelium at the site of abembryonal contact. The slight separation of the ovum from the epithelium is a shrinkage artifact. The presence of

the ovum has induced an obvious change in the subepithelial tissues. There is a notable increase in the size of the nuclei of the stromal cells and their chromatin material is distributed peripherally. The quantity of cytoplasm has increased substantially and stains more basophilically. Relatively few mitotic figures are visible in the "activated" endometrium and those present are largely confined to the rapidly proliferating endothelium of the capillaries. The changes described may be observed only in those antimesometrial areas where the abembryonal poles of the ova are in contact with the uterine epithelium. The rapidly expanding, eccentric growth of the endometrium is wholly responsible for the formation of the crypt in which the ovum is lodged (figs. 4, 7 and 8). The specific details of the epithelial changes at the site of implantation which have been observed here are in accord with those previously described by Krehbiel ('37).

In fresh, pregnant uteri, in which the decidual crypts are slightly in advance of that shown in figure 7, it is frequently possible to dissect out the entire uterine epithelial lining intact. This dissection impressively demonstrates the nipple-like, antimesometrially directed elongations of the uterine epithelium which form the lining of the decidual crypts. From many observations on fresh material one gains the impression that the abembryonal pole of the blastocyst remains tenously fixed in position on the epithelial surface and that as the crypt deepens the ovum is more or less passively carried below the general surface level. This implies that the abembryonal cells have certain adhesive characteristics. There is as yet no evidence to refute the possible presence, in the rat, of protoplasmic extensions of the cells at the abembryonal pole of the sort demonstrated in the Sciuroidea (Mossman, '37), and in the guinea pig (Spee, '01, and Blandau, '49).

The studies on normal implantation in the rat raise two important questions: (1) what is the stimulus which initiates the decidual reaction and, (2) what part does the ovum play in the removal of the epithelial lining of the uterus to com-

plete implantation? The investigations of Alden ('48) on the activity of the primary trophoblast giant cells have answered the second question with considerable conclusiveness. He found that these cells, by their undermining, cytolytic and phagocytic activities, effectively removed the epithelium lining the crypt. It is important to note that although the primary trophoblast giant cells make their appearance in the abembryonal pole during the early stages of decidual crypt formation the lining epithelium does not undergo dissolution until the crypt has reached a considerable depth, even beyond that shown in figure 7.

Decidual responses to "artificial ova." To obtain further information concerning the stimulus which initially activates the endometrium and to determine whether the ovum exerted a specific effect in inducing this reaction an average of 6 glass or paraffin beads, of the approximate size of ova (fig. 2), were inserted into the reproductive tract of 30 pseudopregnant rats as previously described.

Relatively few deciduomata resulted from beads inserted directly into the periovarial space during the period of heat. The slightest bleeding caused the beads to be held locally in fibrin clots. Even though some of the beads remained free they frequently did not find their way into the oviduct and were still found within the periovarial space when the females were killed. If no bleeding attended the insertion of the beads into the dilated loops of the ampulla, within 24 to 36 hours after ovulation, they passed through the oviducts, entered the cornua and induced deciduomata in a manner similar to those formed by normal fertilized ova. The greatest success in inducing deciduomata in the rat resulted from beads which were deposited directly into the uterine lumen by entering it, either through the oviduct just above the utero-tubal junction, or by the direct penetration of the uterine wall near the utero-tubal junction. Figure 3 is a whole mount of a reproductive tract in which, on the third day after the onset of heat, 6 beads were inserted into the left cornu only. This animal was killed on the 6th day. Six deciduomata formed

as a result of the presence of the beads; an additional one, largest of all and located near the utero-tubal junction, was the result of the passage of the pipette through the anti-mesometrial wall.

The gross appearance of the decidual reactions at the time when decidual crypts are forming in both a normally pregnant and an experimental animal may be observed in figures 4 and 5. The specimens were removed from animals killed approximately 5 days after ovulation. In figure 4 a portion of the cylindrically shaped, implanting ovum may still be seen in the crater-like, central depression of the deciduoma. A longitudinal section through another pregnant uterus removed at approximately the same time may be seen in figure 7. The uterus shown in figure 5 was removed from a pseudo-pregnant animal which had not been allowed to mate but in which 4 beads had been inserted into the ampulla of one of the oviducts 24 hours after ovulation. The black bead is partially visible in the center of the decidual crypt. The reaction of the endometrium to the bead appears identical to that of the normally pregnant uterus (fig. 4). More advanced stages of the experimentally induced deciduomata may be seen in figures 9 and 10. In figure 9 the arrow indicates the original position of the bead. The uterine lumen, although greatly reduced, is still continuous with that of the decidual crypt. The decidual reaction is similar to that of the control except that the stromal cells in the immediate vicinity of the bead are somewhat more vacuolated. The extent of the vacuolation of the cells in this area varies from animal to animal in both the control and experimental groups. In figure 10 the bead has become lodged at the very apex of the crypt. The epithelial lining in its immediate vicinity is still intact but the lumen of the crypt is no longer continuous with that of the cornu.

The results of the experimentally induced deciduomata in the rat leave no doubt that inert objects, having the approximate size and shape of blastocysts, can instigate the first stage of implantation by inducing a decidual reaction which resembles in most respects those initiated by the blastocysts.

Epithelial reactions to "artificial ova." Krehbiel ('37) demonstrated that electrical stimulation of a properly sensitized endometrium produced deciduomata "which histologically and cytologically closely simulates the series of changes seen in normal pregnancy," even to the disappearance of the lining epithelium and extravasation of blood. His observations and those of Selye and Mckeown ('35) would thus seem to "militate against the theory of active invasion by the embryo." However, the later observations of Alden ('48) on the removal of the epithelium by the trophoblast giant cells served to emphasize the fact that the tissue destruction could be brought about apparently by both specific and non-specific mechanisms. The observations to be described below, on the later stages of the experimental implantations in the rat will serve to confirm these conclusions.

Figures 11 through 13 are longitudinal sections of decidual crypts removed during the 6th day after ovulation. In a normally implanting ovum at this stage the epithelial lining of the crypt, in contact with the trophoblast cells, is disappearing (fig. 11). The lumen of the narrow crypt is still continuous with that of the cornu. Some of the stromal cells in the vicinity of the ovum contain vacuoles of varying size. The epithelium lining the crypt in tthe experimentally induced deciduoma (fig. 12) is greatly thinned out but intact except for a small area in the immediate vicinity of the "artificial ovum." In this specimen there is extensive vacuolation of the stromal cells and many of their nuclei appear pycnotic and stain intensely. A few leukocytes are present in the lumen of the crypt but there is no evidence of hemorrhage. In contrast, the epithelial lining of the crypt in figure 13 has completely disappeared. Considerable hemorrhage has filled the implantation cavity with blood but there is no evidence of bleeding into the decidual tissue itself. Except for the presence of considerable edema in the tissues bounding the implantation cavity (fig. 13) the general pattern of decidual reaction compares favorably with that of the control. Thus

it is apparent that the epithelial lining of the decidual crypt may be lost as the result of the presence of either a normal or "artificial ovum." The factors causing the disintegration in either case, however, appear to be due to entirely different phenomena.

The sensitivity of the rat's endometrium to foreign stimuli is further emphasized by the following observations on a female rat killed 5 days after mating. The oviducts were greatly distended with purulent material and on opening a section of the uterus it was evident that a large number of decidual crypts, of varying depth, were forming along the antimesometrial border. A longitudinal section through a portion of this uterus (fig. 14) demonstrates the multiple decidual reactions. The source of the stimuli were clumps of leukocytes and bacteria. This may explain the occasional observation in normal and vitamin deficient animals in which the entire cornua of mated females, having infections of the reproductive tract, appear as a single continuous deciduoma (Evans, '28).

Observations on the guinea pig

During preliminary phases of this investigation it was necessary to obtain further information on earlier stages of attachment of the guinea pig ovum than had heretofore been described. The results of these observations have been published elsewhere (Blandau, '49).

As has been pointed out previously, the formation of the decidual reaction in the rat precedes the removal of the epithelial cells lining the crypts by a considerable period of time. In the guinea pig, however, the sequence of events is somewhat different in that the removal of the epithelial lining is preceded by, or occurs simultaneously with, the initiation of the decidual reaction. In an effort to determine whether the reaction of the guinea pig's endometrium to inert objects was similar to that observed in the rat, two to 6 paraffin or glass beads, ranging in size from 60 μ to 175 μ were inserted into

one oviduct or cornu in 46 animals between one to 5 days after ovulation. The animals were killed between the 7th and 10th days following ovulation.

In no instance did the beads induce decidual reactions similar to those observed in uteri containing normally implanting ova, nor was the epithelium lining the uterus penetrated. Even though there was no evidence of a decidual response as the result of the presence of the beads of the approximate size of ova (60 μ to 75 μ), minimal decidual reactions occurred if beads of 150 μ to 175 μ were inserted. These decidual reactions were, however, not at all comparable to those induced by blastocysts, nor was the epithelial lining destroyed or penetrated in any case. The uterine sections shown in figures 15 and 18 were obtained from two animals which had ovulated at approximately the same time. The control animal was allowed to mate. Four paraffin beads (150 μ to 175 μ) were inserted into the lumen of one cornu of the experimental animal on the 4th day. Both were killed on the 7th day. In the guinea pig implantation is completed on the 7th day and the ovum now lies in the subepithelial stroma (fig. 15). The gap in the uterine epithelium, through which the ovum has passed, has been completely repaired. The activity of the parietal trophoblast is causing an active retrogression of the decidua resulting in the enlargement of the implantation chamber. The "artificial ovum" (fig. 18) has induced a minimal decidual response. However, neither the uterine epithelium nor the underlying stroma show any signs of disintegration. In certain uteri containing the larger beads there was a tendency for the beads to become partially enclosed in a depression in the apex of the antimesometrial area. Irrespective of the extent of the enclosure, the continuity of the uterine epithelium was invariably retained. The beads of the approximate size of ova also at times became located in small depressions in the antimesometrial area but they did not effect even a minimal decidual response (fig. 17).

Proteolytic activity of rat and guinea pig ova

The stimulus provided by the ovum in initating the decidual response and effecting implantation is entirely unknown. In comparing the previous observations on implantation of normal and "artificial ova" in the guinea pig, it seemed reasonable to suppose that the implanting ovum produces some ferment responsible for the destruction of the endometrium. Experimental techniques were devised, as previously outlined, in an attempt to either confirm or negate this hypothesis. Guinea pig ova, recovered from the 6th through the 8th day, and rat ova from the 5th through the 6th days, were cultured on various protein substrates as described. The most consistent results were obtained when the ova were cultured in autoplasma or serum on purified gelatin films of 5 μ to 10 μ in thickness.

There were no indications of proteolysis, as noted by the dissolution of the protein films, in individual cultures of more than 50 rat ova. On the other hand, guinea pig ova, cultured under similar conditions, caused a very localized dissolution of the protein substrates.

Immediately before the guinea pig blastocyst makes its attachment to the uterine epithelium the cells of the abembryonal pole penetrate the zona pellucida in a manner similar to that shown in figure 16. A free blastocyst in the stage of development comparable to that described above was cultured for 8 hours in autoplasma on a purified gelatin strip (fig. 19); the control (fig. 20) consisted of a drop of autoplasma alone. An obvious dissolution of the protein occurred at the site of location of the ovum. The location of a 7 day guinea pig ovum, cultured under similar conditions, is shown in figure 21. The ovum at this stage of development appears somewhat pear shaped and, in the photograph, the area of the inner cell mass lies to the left, the attachment pole to the right. This ovum had already implanted and had advanced in its development somewhat beyond that shown in figure 15.

DISCUSSION

Renewed interest and speculation as to the embryo-endometrial interrelationship at the time of attachment have been aroused by the recent observations on the early-implanting human ova (Hertig and Rock, '45), comparable phenomena in the macaque (Wislocki and Streeter, '38; Heuser and Streeter, '41) and the Sciuroidea (Mossman, '37), and the numerous studies on the chemical constituents of the placenta at the time of implantation (Krehbiel, '37; Dempsey and Wislocki, '46; Alden, '47; Wislocki and Wimsatt, '47). It is of greatest importance to recognize that multitudinous variations exist from species to species, not only in the manner in which the decidual reaction is initially induced but also in the mechanism whereby the uterine epithelium and subjacent tissues are removed to make room for the ovum. The inadequacies in our interpretation of implantation phenomena will be largely remedied when accurate information is obtained on the very earliest stages of ovum attachment.

The problem of polarization of the trophoblast at the time of attachment is one of exceeding interest and has been little investigated. This failure is due primarily to the difficulty in obtaining the ova at this critical period. Normally there is a definite and constant relationship in the orientation of the blastocyst to the uterus. In most mammals the area of the inner cell mass is directed toward the antimesometrial side but in the Rodentia and Megachiroptera the reverse is true; that is, it is directed toward the mesometrium (Mossman, '37). The factors involved in determining this orientation are little understood. The meager information at hand seems to indicate that orientation may be influenced by inherent qualities within the trophoblast itself which provides certain of these cells with specialized qualities for adhesion, cytolysis and phagocytosis (Mossman, '37; Wimsatt, '44; Alden, '48; and Blandau, '49). Thus, for example, in the 9-day macaque blastocyst there is active proliferation of certain cells in the region of the blastocyst wall of the embryonal pole which endows them with special properties for the invasion of the

uterine epithelium (Heuser and Streeter, '41). These investigators suggest that the adhesive properties of the blastocyst wall, after the zona pellucida has been lost, "may be part of the implanting mechanism." Many investigators have decribed the stickiness of the zona-free blastocysts in vitro, but this phenomenon is ordinarily not present in ova observed on the surface of fresh uteri of rats and guinea pigs. Under these circumstances unattached blastocysts may be rolled along the surface epithelium without demonstrating these adhesive properties. On the other hand, in both the rat and guinea pig, the abembryonal poles of blastocysts which have already establshed contact with the uterine epithelium demonstrate varying degrees of adhesiveness. The ovum of the guinea pig, even though it has made only a very superficial contact, ordinarily retains its position through the rigors of fixation (Blandau, '49). If the superficial epithelium has been partially invaded by the abembryonal cells, any attempt to remove the ovum results in the blastocyst wall being torn away without loosening the abembryonal pole. On the other hand, in the carefully opened, fresh uteri of the rat, in which the ova have already initiated the decidual response, the abembryonal poles are only tenuously attached. It is the author's opinion that after the ovum has made contact with the uterine epithelium it is passively carried inward as the crypt deepens.

In the guinea pig the protoplasmic processes of the abembryonal cells may make contact with the uterine epithelium even before the zona has been lost. This is the only mammal in which protoplasmic extensions of the abembryonal cells have been shown to actively penetrate the zona pellucida while the ovum is still loose within the uterine lumen (Spee, '01; Blandau, '49). There is inadequate information in all animals except the guinea pig regarding the shedding of the zona and the possible role of the trophoblast in effecting its removal. From the observations of Lee, ('03) and Mossman, ('37) on the early implantation stages of the various genera of ground squirrels and chipmunks there is little reason to

doubt that the same general relationship of the attachment cone cells to the zona exist in these animals. The specialized activities of certain of the trophoblast cells, as mentioned, make it clear that at least in some species the ovum plays an important role in its orientation and initial attachment. This information, however, gives no specific clue as to whether the removal of the uterine epithelium itself is effected by purely mechanical or chemical means.

While investigating the implantation phenomena in Tamias striatus, Mossman concluded that the abembryonic trophoblastic "attachment cones" became anchored by insinuating themselves between the epithelial cells of the lining. Simultaneous with this attachment, edema appears in the tissues immediately below the ovum. He concludes: "The epithelium does not degenerate until its blood supply is interfered with by some change beneath it, usually edema. The epithelium degenerates by becoming itself edemic, the nuclei are crowded away from the base of the cells, vacuoles appear and the cells fragment." The description of this phenomenon in Tamias and other members of the squirrel family would indicate that the edema could be the result of a purely irritational mechanism, or also it might be due to the action of some chemical substance liberated by the trophoblast cells. The ovum in either case is the agressor and the possibility that it may later exercise cytolytic and phagocytic activities needs to be investigated.

The descriptions of the implanting 9-day macaque ovum indicate that the areas of first attachment are situated peripherally in the region of the thickened trophoblast and that the "epithelial implantation placque is clearly a reaction that follows the attachment of the egg and not one that precedes it," (Wislocki and Streeter, '38; Heuser and Streeter, '41). The cytolysis of the cytoplasm and nuclei occurs specifically at these points of invasion. It is important to note that while the surface epithelium is still intact the underlying stroma shows little change, indicating the very restricted area of influence of the trophoblast.

In the guinea pig the abembryonal pole cells also appear to cytolyze the surface epithelium before the underlying stroma shows any detectable change which ordinarily attends the decidual response. In both the macaque and cavia the epithelium seems to act as a barrier which, under normal conditions of pregnancy, must be at least partially removed before the decidual response can be initiated. Quite the reverse situation occurs in the rat and possibly also the mouse. In these animals the decidual reaction progresses to an advanced stage before there is any demonstrable disturbance of the superficial epithelium.

The studies of experimentally induced deciduomata have challenged the earlier beliefs that the ovum is necessary for the complete differentiation of the decidua and that the trophoblast plays an active chemical role in effecting the breakdown of the maternal tissue and converting it into histiotrophe. The observations of Krehbiel ('37) and those presented here clearly indicate the great sensitivity of the rat's endometrium to small stimuli. The slightest disturbance of the superficial epithelium is sufficient to instigate the decidual reaction. It is still not clear in the case of the rat, whether the blastocyst initiates the decidual response by mere contact with superficial epithelium or whether some chemical substance is also excreted by the abembryonal cells which by itself serves as the stimulus. In the rat, the evidence at hand strongly favors localized pressure as being a sufficient stimulus to induce the decidual response; but in the guinea pig the removal of the uterine epithelium is necessary before the decidual response is elicited.

It would be interesting to know whether a very early decidual response, such as that shown in figure 6, would continue to grow and develop maximally if the embryo were removed during this period. The production of deciduomata by momentary electrical stimulation of the surface of the cornua (Krehbiel, '37) lends partial support to the belief that once a stimulus has been applied to the endometrium it acts more or less as a catalyst which causes the "endometrial

reaction'' to go to completion. But even with instantaneous electrical shock there is no way of knowing the amount of alteration or damage to the cells in the area stimulated.

The loss of the epithelium lining the endometrial crypts may occur both in the presence and absence of the ovum. If the ovum is present the epithelium is lost due to the specific activities of the trophoblast giant cells. If an ''artificial ovum'' or even no ovum is present in a properly stimulated endometrium, the epithelium is lost as the result of edema and probably also ischemia in the subjacent stroma. The great variability in the manner in which this epithelium is lost under experimental conditions further bespeaks the many factors which may effect its dissolution.

The failure of the rat ova to demonstrate proteolysis in vitro may have been due to the technique used. Since the trophoblast giant cells form a syncytium with the epithelium lining the crypt and possibly also with the subjacent cells, they may not have been removed with the ovum. On the other hand, since active phagocytosis is a prominent feature in the removal of the epithelium it is possible that cell digestion may occur only within the substance of the trophoblast cells themselves.

In the guinea pig, the failure of the ''artificial ova'' to remove the uterine epithelium and the ability of the cultured ova to effect proteolysis in vitro strongly suggest that the implanting ova elaborate proteolytic ferments. Further investigations are in progress to determine whether ''artificial ova,'' containing proteolytic enzymes which are gradually released from them in utero, have the ability to digest the uterine epithelium and effect implantation in this animal.

SUMMARY

Paraffin and glass beads, of the approximate size of ova, were inserted into the reproductive tracts of pseudopregnant rats and guinea pigs at varying intervals after ovulation, and their effects on the endometrium noted. In addition, implanting rat and guinea pig ova were cultured on various protein

substrates in order to determine whether or not they could effect proteolysis in vitro.

1. Beads inserted into the reproductive tracts of rats initiated decidual reactions which were identical, in their rate of formation and histologic appearance, to those induced by normal fertilized ova.

2. In the guinea pig the beads induced no decidual reactions similar to those observed in uteri containing normally implanting ova, nor was the epithelium lining the uterus penetrated.

3. Minimal decidual reactions were observed in the guinea pig when beads of approximately two to three times the size of blastocysts were inserted into the uterus.

4. Guinea pig ova, recovered 6 to 8 days after fertilization and cultured on various protein films, caused proteolysis of the substrates. Rat ova, recovered on the 5th and 6th days after fertilization, failed to cause proteolysis when cultured under similar conditions.

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PLATE 1

EXPLANATION OF FIGURES

2 Comparison of the sizes of "artificial ova" (glass and paraffin beads) with normal, living two-cell rat ova. Photographed in a hanging drop of Ringer's solution. × 100.

3 Whole mount of the reproductive tract of a pseudopregnant rat killed on the 6th day after ovulation. Six "artificial ova" had been inserted into the lumen of one cornu on the third day. Seven individual deciduomata are present. Beads were recovered from 6 of the smaller ones. The large decidual response, near the ovary, was caused by the passage of the pipette directly through the antimesometrial wall. × 1.

4 Whole mount of uterus cut along the mesometrial border from a mated rat killed on the 5th day of pregnancy. Note the early decidual reaction in the antimesometrial area with the ovum partially visible in the center of the crater-like depression. × 15.

5 Whole mount of uterus removed from an unmated but pseudopregnant rat killed approximately 5 days after ovulation. The "artificial ovum" seen in the crater-like depression of the deciduoma had been inserted into the ampulla of the oviduct 24 hours after ovulation. × 15.

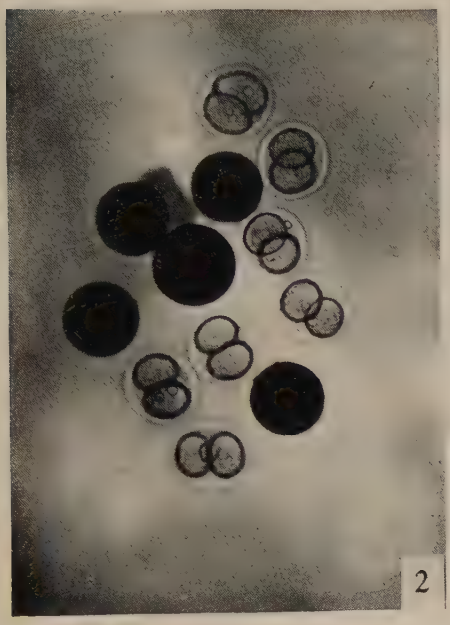
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PLATE 2

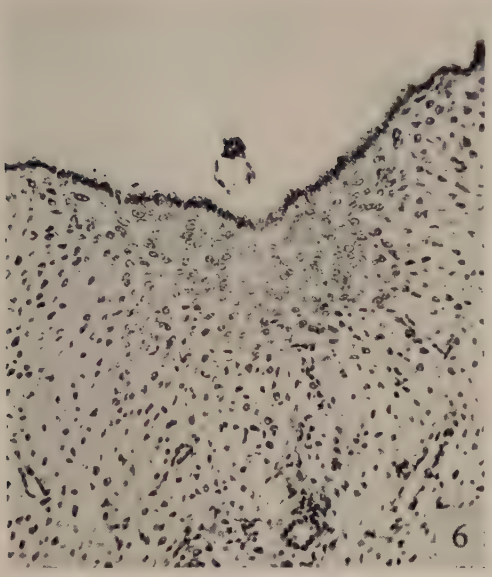
EXPLANATION OF FIGURES

6 Longitudinal section through the antimesometrial wall of a pregnant rat killed on the 5th day. As yet there is no visible change in the superficial epithelium at the site of ovum contact. There is, however, a rapid change in the subepithelial stroma involving both the decidual cells and capillaries. × 140.

7 and 8 Longitudinal sections through decidual crypts of pregnant rats killed on the 5th and 6th days, respectively. The rapid, eccentric development of the decidua is responsible for the formation of the crypts. There is considerable shrinkage of the ovum in figure 7. The ovum completely fills the crypt in the fresh specimens of this same age. × 15.

9 Experimentally induced deciduoma resulting from the presence of paraffin bead the approximate size of an ovum. The rat was killed on the 5th day. The arrow points out the location of the bead. The general decidual response and formation of the crypt is identical to that of the control. Epithelium of the crypt is greatly thinned out but intact. × 15.

10 Section of a uterus of a pseudopregnant rat killed on the 6th day. The bead is located in the apex of the crypt. The epithelial lining of the crypt is exceedingly thin but intact. There is, however, no continuity between the lumen of the crypt and cornu. × 15.



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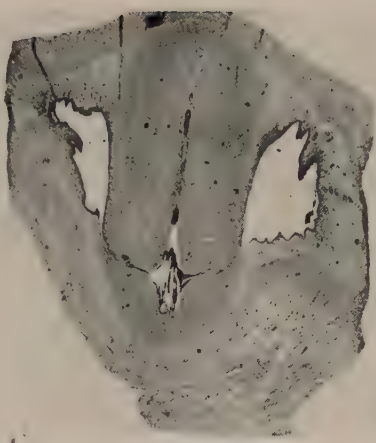

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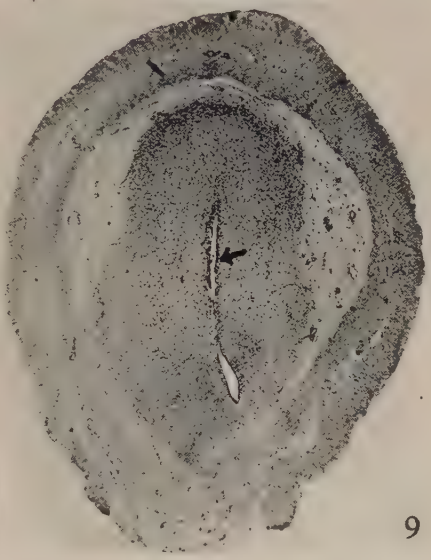

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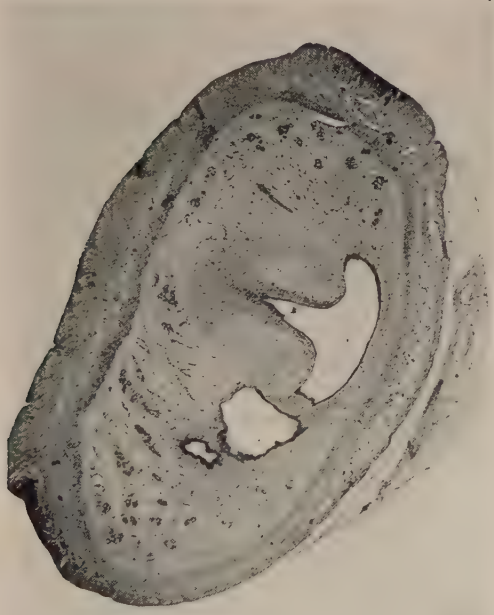

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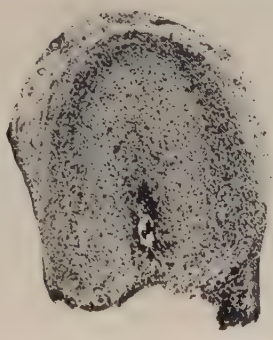

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PLATE 3

EXPLANATION OF FIGURES

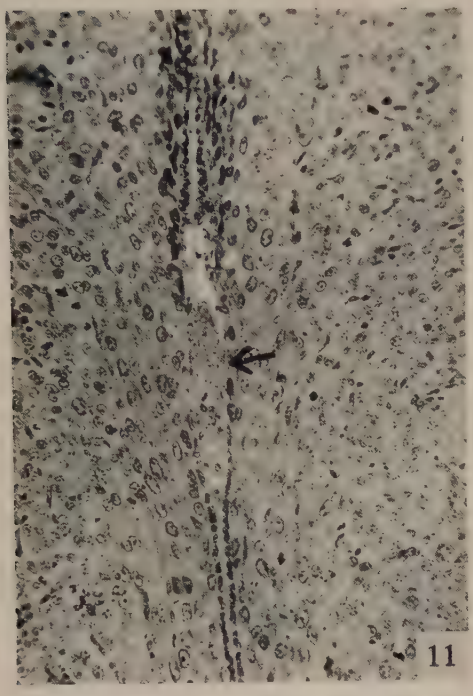
In all photomicrographs on this plate the mesometrium is toward the bottom of the plate.

11 Arrow points out the location of a normally implanting 6-day rat ovum. The epithelial lining of the crypt in the immediate vicinity of the ovum is disappearing due to the activities of the trophoblast giant cells. × 150.

12 Section through a decidual crypt in an experimental animal also killed on the 6th day. Arrow points out the remains of the lamp black, the original position of the paraffin bead. The epithelium lining of the crypt is very thin but intact except for that immediately adjacent to the "artificial ovum." The lumen of the crypt contains a few leukocytes but there is no evidence of hemorrhage. The cells of the stroma show considerable vacuolation when compared to figures 11 and 13. × 150.

13 Section through a decidual crypt of an experimental animal killed on the 6th day. Arrow indicates the position of the glass bead. The epithelium lining the crypt has disappeared completely and hemorrhage has occurred into the implantation cavity. Except for an increase in edema in the stroma bounding the implantation cavity the general decidual reaction is very similar to the control (fig. 11). × 150.

14 Longitudinal section through a uterus of a mated animal with salpingitis, killed early on the 5th day. In place of implanting ova decidual crypts were found which varied in depth and which resulted from the stimulus provided by clumped masses of leukocytes and bacteria. × 15.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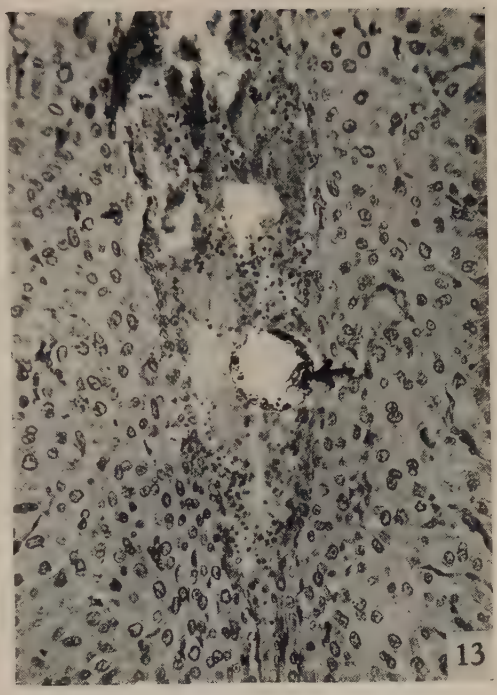
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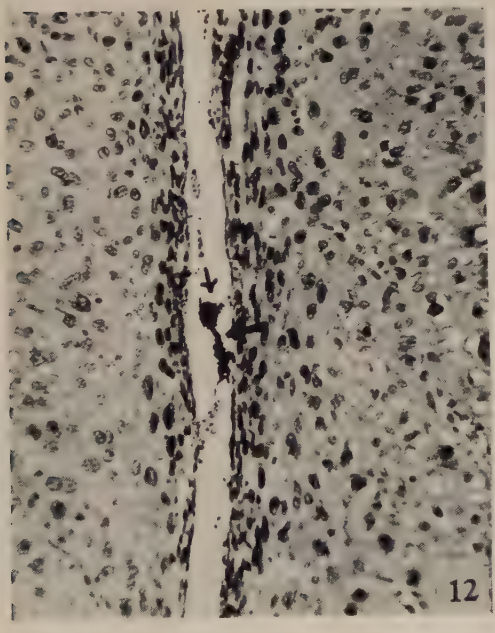
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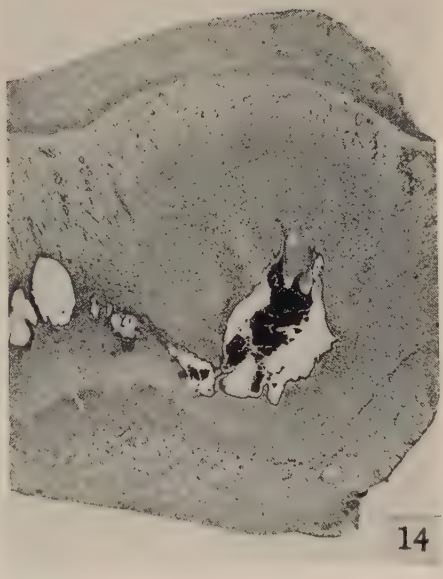
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PLATE 4

EXPLANATION OF FIGURES

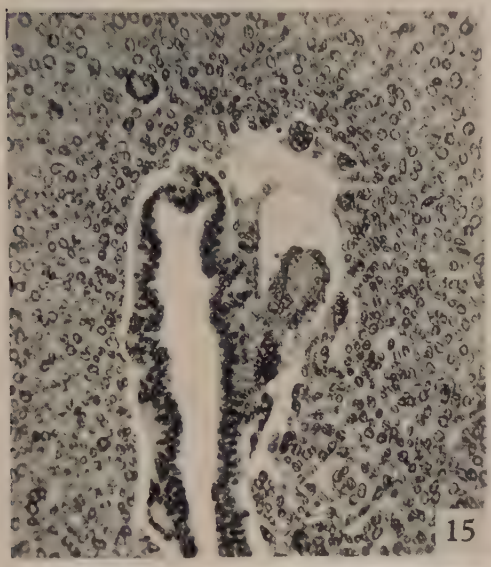
15 Normally implanted guinea pig ovum removed from a female killed on the 7th day after ovulation. The ovum is completely embedded in the subepithelial tissues and the implantation cavity is rapidly enlarging. The mesometrium is toward the bottom of the plate. × 150.

16 Hanging drop preparation of a living guinea pig ovum recovered during the 6th day. Note the cytoplasmic processes penetrating the zona pellucida in the region of the abembryonal pole, and their extensions beyond the zona. Blastocysts examined immediately upon removal ordinarily completely fill the perivitelline space. × 450.

17 and 18 Sections of uteri containing "artificial ova" from animals also killed on the 7th day. In figure 17 the glass bead of approximately 70 μ in diameter shattered during sectioning but more or less retained its original position. There is no evidence of a decidual response. In figure 18 a larger paraffin bead (150 μ to 175 μ) had induced a minimal decidual response but the epithelial lining is intact. The mesometrium in both instances is toward the bottom of the plate. × 15 and 150.

19 and 20 Examples of dissolution of thin, purified gelatin strips as the result of the presence of an ovum cultured in autoplasma in the stage of development shown in figure 16. Figure 20 is a control gelatin strip on which a drop of autoplasma alone was cultured.

21 Dissolution of a purified gelatin strip due to the presence of a 7-day guinea pig ovum cultured in autoplasma. The region of the inner cell mass is toward the left.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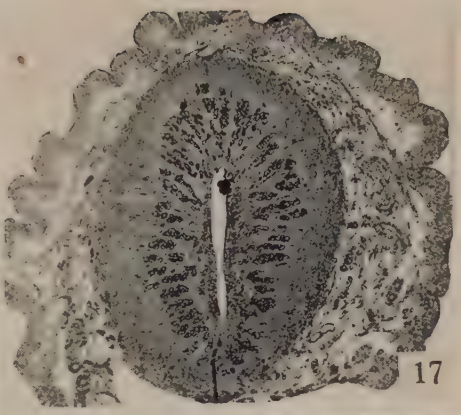
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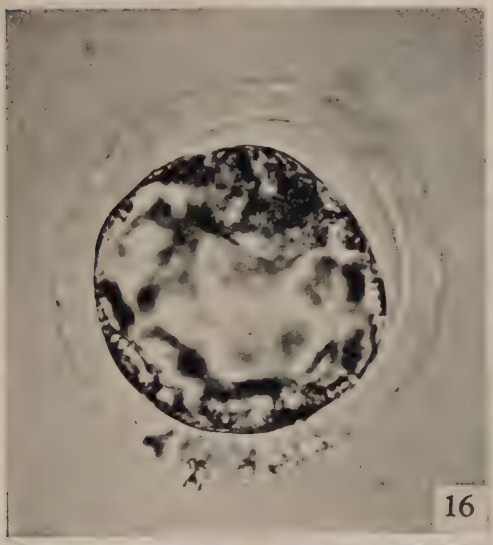
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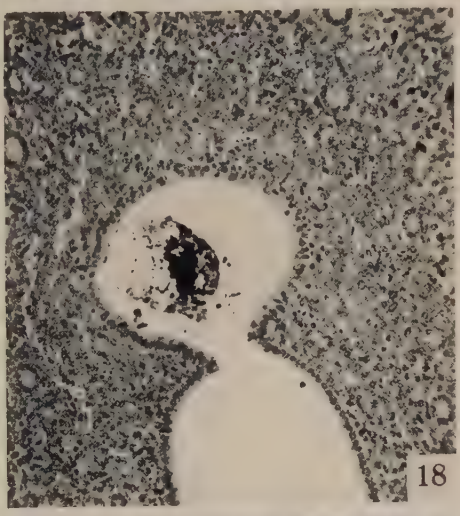
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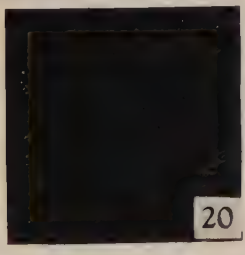
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21

THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE GENITAL TRACT OF MALE MAMMALS [1]

HOWARD A. BERN
Department of Zoology, University of California, Berkeley

FOURTEEN FIGURES

Sex accessories which are morphologically and embryologically homologous in a series of male mammals have been shown to differ considerably in certain of their physiologic activities (*e.g.*, Engle, '26; Humphrey and Mann, '48). Biochemical studies have revealed differences in the occurrence of acid and alkaline phosphatases in the prostate complex and other accessory glands in several mammalian species (Gutman and Gutman, '38; Huggins and Webster, '48; Bern, '49). The present histochemical study of the genital tract of male mammals was undertaken to determine the localization of alkaline phosphatase in specific tissue areas and to investigate any possible relationship between this enzyme and other known components of sex accessory secretions.

Histochemical evidence of alkaline phosphatase activity in certain male sex accessories has been reported previously by Gomori ('41b), Kabat and Furth ('41), Montagna and Noback ('46), Zorzoli and Stowell ('47), Atkinson ('48), Soulairac and Thibault ('48), Dempsey, Greep and Deane ('49) and Wislocki ('49). Acid phosphatase has been demonstrated by Gomori ('41a), Wolf, Kabat and Newman ('43) and Wislocki ('49). However, we have not found any detailed comparative study in the literature dealing with the male genital tract.

[1] Aided by a research grant from the University of California Board of Research.

MATERIALS AND METHODS

The genital tracts of 58 sexually mature male mammals were examined for the differential occurrence of alkaline phosphatase in fibromuscular tissue, subepithelial stroma, epithelium and secretory product. Six mice, 15 rats, 15 Dutch rabbits, 8 guinea pigs, 5 golden hamsters, 4 bats *(Corynorhinus rafinesquii intermedius)* and 4 opossums *(Didelphis virginiana)* were employed. All animals, including bats and opossums, showed large, actively secretory, accessories. Organs studied included seminal vesicle, vas deferens, cranial and caudal epididymis, testis, several lobes of the prostate complex, bulbourethral gland, bladder and urethra, as well as preputial gland in the rat and mouse.

Portions of these tissues were fixed in chilled acetone (and occasionally in chilled 80% alcohol) and treated by the Barger ('47) modification of the Gomori technic. Tissues from some rabbits, rats and guinea pigs were stored in the acetone of fixation for three months before further treatment, and no significant alterations in enzyme localization were noted. The phosphatase determined is an alkaline glycerophosphatase. Duration of incubation in the substrate at about pH 9.4 and 37°C. ranged from two to 24 hours in the initial stages of this investigation; however, 18 hours' incubation was chosen as the basis for the comparative study.

It was noted that the heaviness of the reduced silver precipitate was dependent on the duration of time of washing in distilled water after incubation. Longer washing ($\frac{1}{4}$–$\frac{1}{2}$ hour) did not change the localization of the enzyme but made the slides considerably more desirable for detailed histologic study, although less differentiated for photomicrography. A light hematoxylin stain was added to the sections.

Controls for each slide were carried through the entire technic, using an identical substrate poisoned with approximately 0.01 M KCN (Lison, '48). In standing solutions this concentration was occasionally insufficient to inhibit all phosphatase activity in the apical borders of certain epithelial cells (epididymis, seminal vesicle) and in the basement mem-

brane region. Replacement of the KCN was thus necessary in solutions used more than once. Emmel ('46) has reported a lower sensitivity of intestinal and kidney tubule alkaline phosphatase to KCN poisoning in these areas (see also Gomori, '49).

OBSERVATIONS

The locations of the principal sites of alkaline phosphatase activity are indicated in table 1. The prostate is not included therein due to the difficulty in establishing definite homologies between the various lobes in the several species. The comparisons of enzyme activity reported below are based on cytoplasmic and extracellular evidences. Nuclear alkaline phosphatase was only occasionally apparent in the sections studied, although Danielli and Catcheside ('45) stated that all vertebrate cell nuclei contain some alkaline phosphatase activity.

Testis. The testes of all animals except the hamster show alkaline phosphatase activity in the cytoplasm of the developing sperm cells. The later sperm stages show greater activity than the earlier stages in the guinea pig and mouse; this is especially evident in the cytoplasmic remnants in the lumina.

The basement membrane of the seminiferous tubules is strongly active in all testes, except those of the rabbit and bat. The interstitial cells rarely show limited activity.

Epididymis. The epithelium of the epididymis varies in activity in different regions. Thus, in the cranial epididymis of the rat (fig. 3) it is generally positive (as is the sperm-secretion mass); whereas in the caudal epididymis (fig. 2) the epithelium and sperm mass are negative. The reverse picture obtains in the hamster and opossum. The stroma is universally positive (figs. 2–3), except for occasional areas in the rabbit's cranial epididymis. When the epithelium is positive, the strongest reaction is seen in the stereocilia (apical) region of the cells.

Vas deferens (fig. 1). The fibromuscular wall of the vas is strongly positive in the mouse (fig. 14) and rat (fig. 10) and very slightly in the guinea pig (fig. 12); it is inactive in

TABLE 1

Distribution of alkaline phosphatase in male urogenital tracts

	RAT	MOUSE	HAMSTER	GUINEA PIG	RABBIT	BAT	OPOSSUM
Testis							
Spermatic epithelium	+	+	−	+	+	+[1]	+
Basement membrane	+	+	+	+	±	−	+
Intertubular stroma	±	±	−	−	±	−	±
Cranial epididymis							
Epithelium	+ or −	+	−		+	+	−
Sperm-secretion mass	+	+	±		+	+	−
Subepithelial stroma	+	+	+		+ or −	+	+
Caudal epididymis							
Epithelium	−	+	+	−[2]	+	+	+
Sperm-secretion mass	−	+	+	+	+	+	−
Subepithelial stroma	+	+	+	+	+	+	+
Vas deferens							
Epithelium	−	−	+	−	+[3]	−[3]	−
Subepithelial stroma	+	+	+(P)	±(P)	+	+	±(P)
Fibromuscular wall	+	+	−	±(P)	−	−	+[4]
Seminal vesicle							
Epithelium	−	−	−	+	+ or ± (P)		
Secretory mass	−	−	−	+(P)	+[5]		
Subepithelial stroma	+	+	±(P)	−	−		
Fibromuscular wall	+	+	−	±(P)	−		
Bulbourethral gland							
Acinar epithelium	+	+	−	±(P)	+(P)		−
Interacinar stroma	+	+	+(P)	−	+		−
Preputial gland							
Acinar epithelium	+	+(P)					
Ductal epithelium	−	±(P)					
Stroma	−	−					
Bladder							
Epithelium	+	+	±[7]	+[7]	+[7]	−	−
Subepithelial stroma	−	+	−	+	+	−	+(P)[6]
Fibromuscular wall	−	+(P)[8]	−	−	−	−	−
Urethra							
Epithelium	−	−	+(P)	−	−		
Subepithelial stroma	+	+	+	+(P)	+(P)		

Legend: + = strong alkaline phosphatase activity
± = slight alkaline phosphatase activity
− = no alkaline phosphatase activity
(P) = patches of alkaline phosphatase activity

[1] Seminiferous tubules atrophic
[2] No cranial epididymis in guinea pig
[3] Including ampullae
[4] Activity confined to outermost layer
[5] When present
[6] Activity confined to capillaries
[7] Basal layers only
[8] Stroma between smooth muscle bundles

the bat, rabbit and hamster, except subepithelially. The epithelium is positive only in the rabbit (including the ampullary region) and hamster (figs. 11 and 13).

Seminal vesicle (fig. 1). The seminal vesicle epithelium is consistently strongly positive in the guinea pig (fig. 7). In the rabbit, the gland frequently shows a strong epithelial reaction (fig. 8), but occasionally only patches of slightly active cells are seen. The histochemical observations on the rabbit seminal vesicle agree with previously obtained biochemical

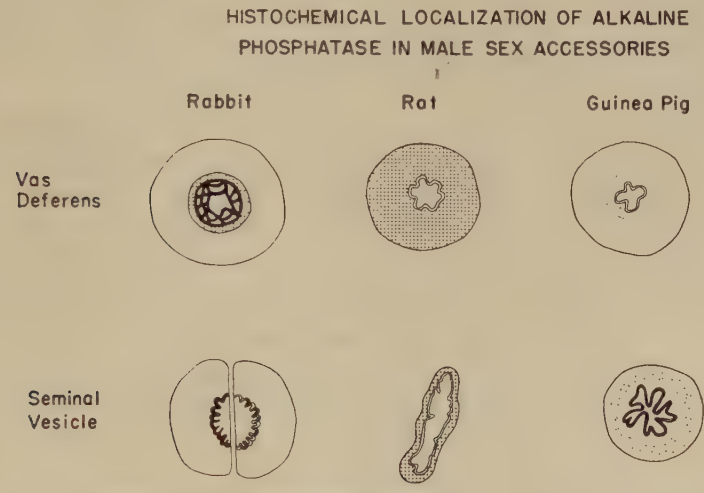

Fig. 1 Diagram to show differences in alkaline phosphatase distribution in the vas deferens and seminal vesicle of the rabbit, rat and guinea pig. Stippled areas indicate location of stromal phosphatase, shaded areas location of epithelial phosphatase.

data (Bern, '49). In the rat (fig. 6) and mouse, the epithelium is negative, but the fibromuscular wall is strongly positive. Some slight activity is also apparent in the smooth muscle in the guinea pig.

Prostate. The several lobes of the prostate complex in a single species may differ greatly in their alkaline phosphatase activity. In agreement with published biochemical studies, the secretion and epithelium of the ventral prostate in the rat and mouse are found to be strongly active, whereas those

of the anterior and dorsal lobes are virtually negative (figs. 4–5). On the other hand, the stroma is strongly positive in the latter and shows some activity in the former. In the bat, the epithelium and secretion of the prostate complex are also strongly positive. In the other animals examined, activity is confined largely to the subepithelial or interglandular stroma, most often associated with capillary endothelium (fig. 9).

Bulbourethral gland. The acinar epithelium of the bulbourethral gland shows some activity (often quite variable) in all species except the hamster and opossum, and the stroma is positive to some extent in all except the guinea pig and opossum.

Preputial gland. The preputial gland shows enzyme concentrations throughout the epithelium in the rat, but only in certain patches of cells in the mouse.

Bladder and urethra. The bladder epithelium in all animals except the hamster, bat and opossum shows considerable enzyme activity, and the subepithelial region of the submucosa is positive in the mouse, rabbit and guinea pig. Of those animals wherein the urethra (membranous) was studied, the epithelium was found to be slightly positive only in the hamster, but the subepithelial stroma was positive in all.

DISCUSSION

Using the Gomori technic, Soulairac and Thibault ('48) have recently reported that the vas deferens, seminal vesicle, prostate and bulbourethral gland of the rat show alkaline phosphatase activity, but that the testis and cranial and caudal epididymis are completely negative. Our data disagree with these latter observations; however, inasmuch as the duration of incubation and other technical details are not given in their published report, we are unable to account for the differences. Zorzoli and Stowell ('47) also remarked on the strong glycerophosphatase activity of the subepithelial connective tissue in the rat epididymis. On the other hand, our observations on activity in the rabbit and guinea pig

testis and bladder epithelium agree with those of Gomori ('41b) based on one and one-half hours' incubation.

At least two different areas of phosphatase activity can be distinguished in the organs studied: (1) the phosphatase produced as a part of the secretion of an accessory, evidenced as strong activity in the epithelium as well as in the secretory mass, *e.g.*, in the seminal vesicle of the guinea pig and of some rabbits, in the ventral prostate of the rat and mouse, in some epididymidal regions of most species, etc.; and (2) the phosphatase concentrated in the subepithelial stroma and often associated with capillary endothelium. The secreted phosphatase may dephosphorylate organic phosphates in the semen (in the same way that prostatic acid phosphatase splits phosphorylcholine in human semen, according to Lundquist, '47); the stromal phosphatase may play a role in the transport of sugars and related substances into the epithelium, wherefrom they may then be released into the seminal fluid. It is difficult to suggest at this time special functions for other localizations of alkaline phosphatase, such as in the fibromuscular wall of the rat vas deferens.

The possible role of alkaline phosphatase in the transport of fructose into the seminal fluid was suggested also by Soulairac and Thibault ('48). Wilmer ('44) has discussed the importance of the phosphorylation-dephosphorylation mechanism in sugar transport, and Danielli ('46) has considered the possible extracellular activity of alkaline phosphatase in dephosphorylating organic phosphate esters before reabsorption of sugar from kidney tubules.

Mann ('46), Davies and Mann ('47) and Humphrey and Mann ('48) have demonstrated that fructose and citric acid are produced by different sex accessories in different species of male mammals. In addition, ascorbic acid is produced by the seminal vesicle in man and the guinea pig (Berg, Huggins and Hodges, '41). In the rat, the primary sources of fructose are the anterior prostate (coagulating glands), the "median prostate" and the dorsal prostate. We have shown that both anterior and dorsal prostates are rich in stromal phosphatase.

The ventral prostate, on the other hand, which is rich in epithelial phosphatase, produces no fructose but the bulk of the citric acid.

In the rabbit, the prostate complex, seminal vesicle and ampullae are the major sources of fructose, whereas the seminal vesicle is rich in citric acid. The seminal vesicle and ampullae are shown herein to be rich in epithelial phosphatase; the prostate complex shows some stromal activity. However, in both rabbit and rat, the testis and epididymis, containing stromal phosphatase, are evidently almost devoid of fructose (Mann, '46).

The correlation of the various data on composition and source of seminal secretions is complicated by the confusion in anatomic nomenclature and by the incompleteness of the considerable data on accessories in most animals. This latter can be rectified by carrying out identical assays on a series of mammals. Mann ('48, personal communication) has correctly pointed out that organs which are homologous anatomically or of similar embryonic origin may not be endowed necessarily with similar chemical activities.

The lack of a physiologic picture consistent with anatomic homologies is of great interest, even though absence of alkaline phosphatase in certain tissue areas does not eliminate the possibility of acid phosphatase activities in these areas. We have carried out some histochemical studies on the distribution of genital acid phosphatase, but the technic for its demonstration is considerably less reliable than that for the alkaline enzyme (Lison, '48). Consistent pictures of acid phosphatase activity in the same tissue are difficult to obtain, and variations in its distribution must be interpreted with caution. Thus, although alkaline phosphatase is the enzyme regularly associated with the hexose dephosphorylating mechanism, it is not possible at this point to indicate the definitive role of this enzyme in the several genital sites in which it has been demonstrated.

SUMMARY

Alkaline phosphatase has been demonstrated histochemically in the genital tract of 7 species of male mammals. Various genital organs which are anatomically homologous in this series of mammals may show widely different localizations of alkaline phosphatase. Based on the histologic localization of the enzyme, distinction is made between stromal phosphatase and secreted (epithelial) phosphatase, and a possible relation of the former to sugar transport is discussed.

Epithelial and secretory evidences of alkaline phosphatase are especially noticeable in the seminal vesicle of the guinea pig and many rabbits; in the vas deferens of the rabbit (including the ampullae) and hamster; in the ventral prostate, preputial gland and bulbourethral gland of the rat and mouse; and in the prostate complex of the bat.

ACKNOWLEDGMENTS

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PLATES

EXPLANATION OF PLATES

Unless otherwise indicated, these photomicrographs were made from 6 μ sections of acetone-fixed tissues, incubated for 18 hours at 37°C. in sodium glycerophosphate at pH 9.4. A hematoxylin stain has been added and is largely responsible for the nuclear reaction.

PLATE 1

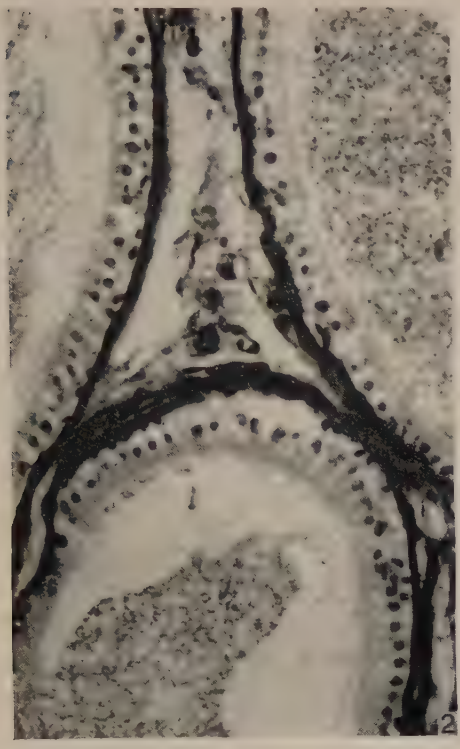
EXPLANATION OF FIGURES

2 Caudal epididymis of rat. Note intense stromal phosphatase activity and negative epithelium. × 250.

3 Cranial epididymis of rat. Note definite epithelial and sperm mass phosphatase activity. × 250.

4 Dorsal prostate of rat. Note intense stromal phosphatase activity and inactive epithelium and secretion. × 250.

5 Ventral prostate of rat. Note phosphatase activity in stroma, epithelium and secretion. *cf.* figure 4. × 250.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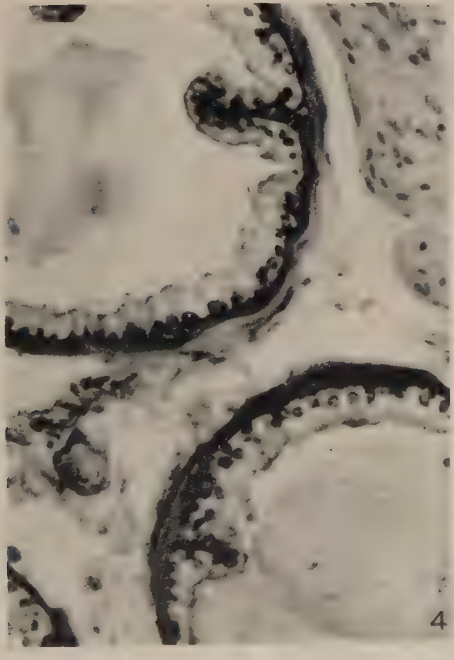
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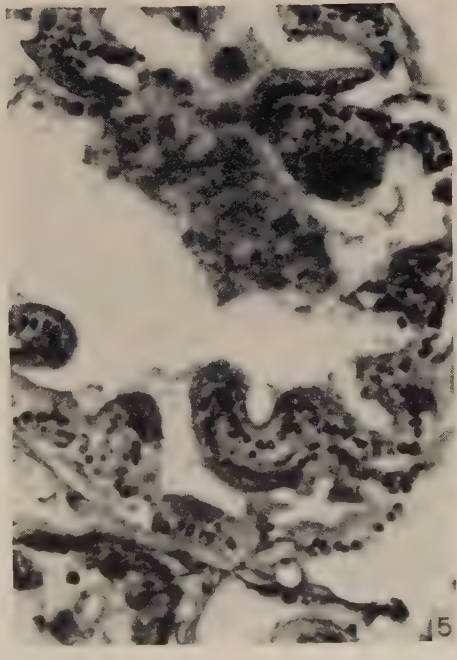
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PLATE 2

EXPLANATION OF FIGURES

6 Seminal vesicle of rat. Note absence of phosphatase activity in secretion and epithelium; intense reaction of subepithelial stroma and fibromuscular wall. × 60.

7 Seminal vesicle of guinea pig. Note intense phosphatase activity of epithelium and secretion; patches of activity in fibromuscular wall. × 60.

8 Seminal vesicle of rabbit. Note intense phosphatase activity of epithelium; absence of activity in fibromuscular wall. × 60.

9 Vesicular gland (prostate complex) of rabbit. 80% alcohol fixation. Note phosphatase activity of subepithelial stroma in association with capillary endothelium. × 250.

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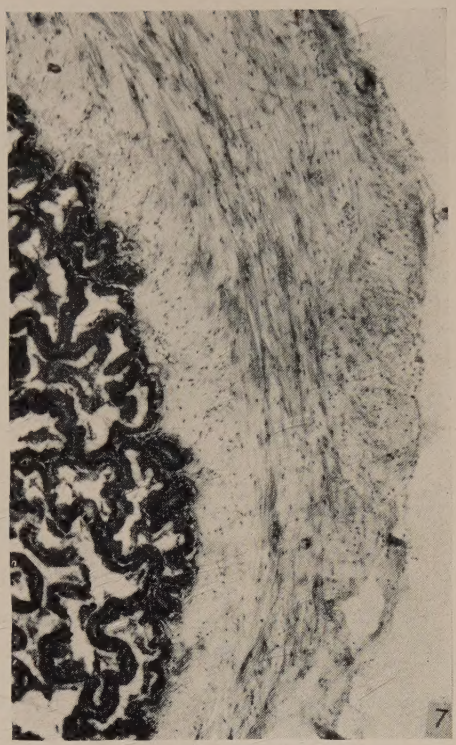
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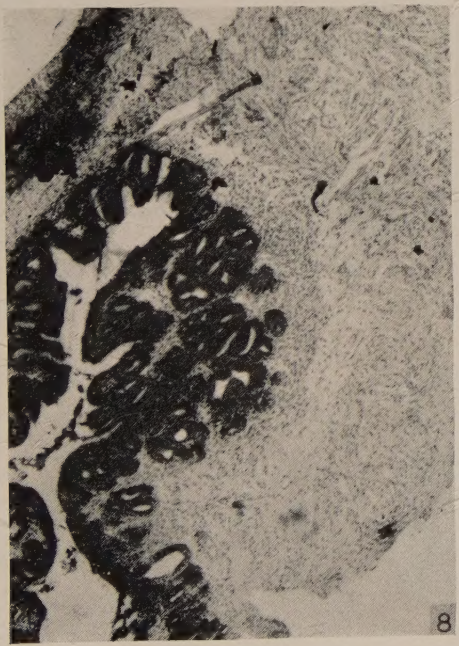
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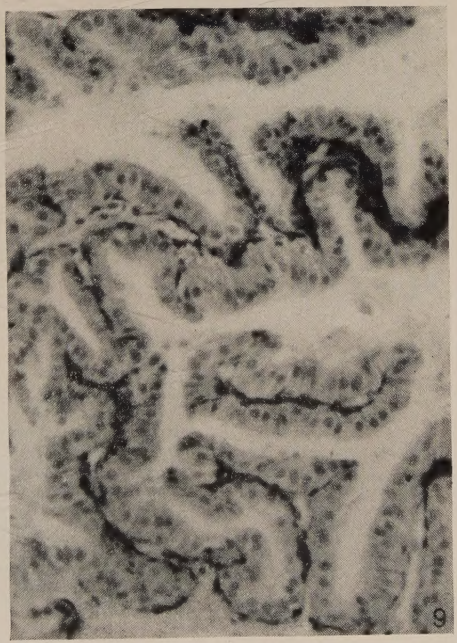
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PLATE 3

EXPLANATION OF FIGURES

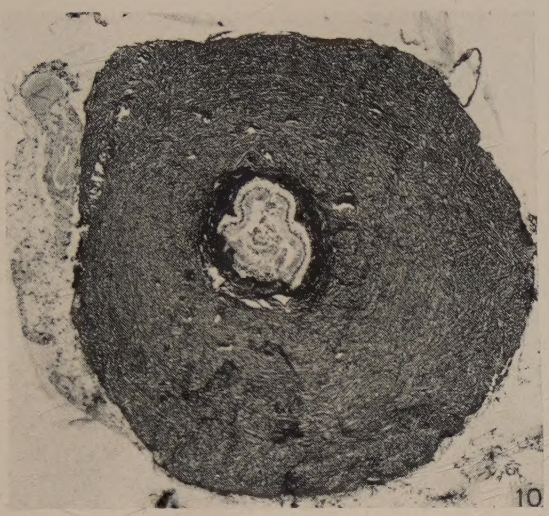
10 Vas deferens of rat. Note intense phosphatase activity of subepithelial stroma and fibromuscular wall; epithelium is negative except for stereocilia. × 25.

11 Vas deferens of hamster. Note phosphatase activity of epithelium and subepithelial stroma, extending into innermost layers of the muscularis. × 25.

12 Vas deferens of guinea pig. Note patches of slight phosphatase activity in fibromuscular wall. × 25.

13 Vas deferens of rabbit. Same picture as in hamster (fig. 11), but more intense. × 25.

14 Vas deferens of mouse. Same picture as in rat (fig. 10). × 25.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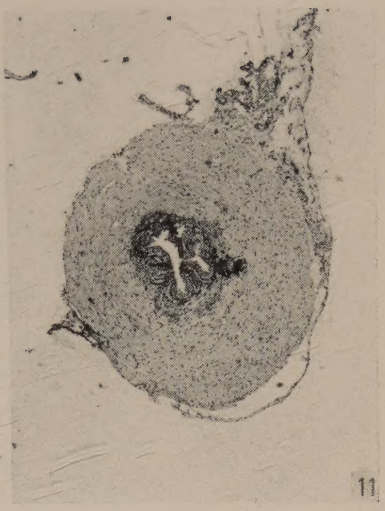
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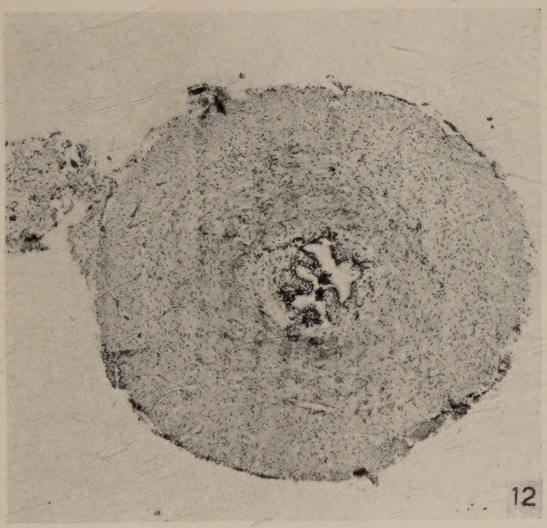
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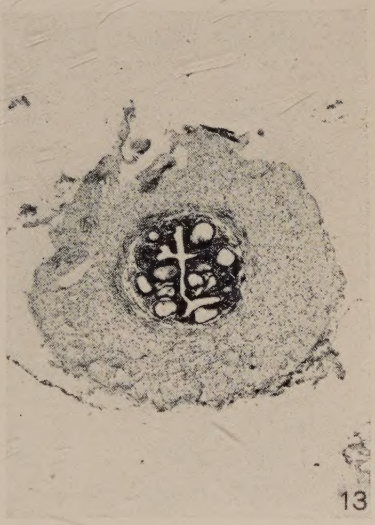
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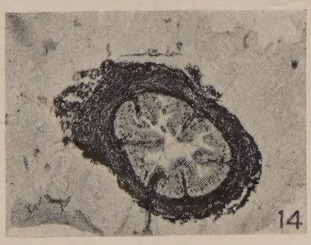
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